Into the Night: Evaluating Sleep as a Measure of Animal Welfare

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Acknowledgments

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Abstract

There is a need for a simple non-invasive measure of animal welfare. In humans, sleep quality correlates strongly with a person's wellbeing; this suggests that sleep may also prove to be a suitable tool to measure mammals' welfare. Studies in humans have shown that lack of sleep compromises the health of individuals, causing heart attacks, strokes, diabetes and even cancer. Likewise, research conducted with shift workers demonstrated they are more likely to develop such diseases but are also more susceptible to psychological conditions such as depression. Considering humans and mammals have similar physiology and sleeping patterns, disturbances in mammals' natural sleeping cycles could have similar outcomes. Our model system to examine this is the domestic dog. This system was chosen because dogs are a well-studied species regarding their physiology and have been used as a model in human sleep studies. They also co-exist with humans which gives us insight on their environment. This thesis presents the results of a multidisciplinary approach to evaluate sleep as a measure of animal welfare in domestic dogs. Firstly, trough behavioural observations, the sleep structure of kenneled dogs was investigated and after finding the dogs had an altered sleep architecture and highly fragmented sleep in the surveyed environment, we then verified the impact of sleep loss in other behaviours. Secondly, using glucocorticoids levels and assessing environmental variables such as temperature, light and sound levels, we evaluated how the environment along with stress responses can further compromise sleep and found important correlations between these measures. Thirdly, using wearable technology, dogs sleep, activity and health parameters (heart rate and respiration rate) were measured and results compared which sleep parameters, demonstrating remote sensing is a reliable technology and can provide further information on the effects of sleep loss in dogs. Lastly, an autonomous system was developed which combines deep leaning techniques (convolutional neural networks) with classical data processing methods to automatically detect and quantify dogs' sleeping patterns and the results demonstrated it is an efficient tool to measure sleep and a practical solution to common problems associated with welfare research.

Keywords: animal welfare, sleep behavior, sleep quality, domestic dogs.

Chapter 1. General Introduction

The development of new methods of assessing animal welfare are ongoing for two main reasons: first, existing methods have scientific limitations and second, many methods are not suitable for everyday use due to logistical problems (i.e. time consuming or invasive). Thus, there is a need for animal welfare assessment that overcomes these two problems, which can be applied across a broad range of species.

1.1 Animal welfare

Presently, the most common definition of animal welfare states that the welfare of an individual depends on its ability to overcome suboptimal conditions in its environment to maintain its physical and psychological health (Broom, 1991; Broom & Molento, 2004; Fraser et al., 1997). Moreover, this is directly associated with the quality of the environment, and the type of response (positive or negative) the individual experiences daily. If the individual repeatedly fails to cope with negative stimuli from the environment, it will start to experience poor welfare. This will be reflected in changes in the animal's behaviour, immune responses and will trigger stress responses, which can further compromise its biological functioning and lead to depressive states (Broom, 1991; Broom & Molento, 2004; Mason, 1991; Mason & Latham, 2004).

1.2 Animal welfare assessment

Evaluating animal welfare can be challenging, since individuals produce responses based on their own ability to cope with the environment and these differences in an individual's response can vary from species to molecular levels (Hill & Broom, 2009; Mason & Mendl, 1993).

Usually, animal welfare studies focus on assessing environmental conditions that may compromise well-being, while monitoring the subject to detect biological variation (e.g., behaviour, physiology) that could be associated with its ability to cope (Hemsworth, Mellor, Cronin, & Tilbrook, 2015; Hill & Broom, 2009).

Common parameters used to assess an individual's responses to its environment involve physiological measurements – such as heart rate (especially its variability), body temperature and stress hormones levels. The evaluation of the behaviour of animals – especially the expression of abnormal behaviour is also frequently used to assess animal wellbeing (Dawkins, 2004; Hemsworth et al., 2015). The occurrence of abnormal behaviours is, often, directly related to failures by the animal to cope with multiples stressors in its environment (Mason & Latham, 2004).

Spruijt & Van Den Bos (2006) proposed that stress "exaggerates" the responses of animals against an adverse stimulus, and even though stress is a natural response for organisms, the recurrence of stressful events can lead to pathologies associated with physiological and neurological attempts to maintain homeostasis (Eriksen & Ursin, 2002).

Animals experiencing negative stimuli beyond their control tend to develop multiple pathologies such as gastric ulcers, hypertension, heart failure, immune system depletion, and have chemical changes in their brains that can lead to emotional changes and depressive states (Babiuk, 2002; Eriksen & Ursin, 2002; Ursin & Eriksen, 2010).

Many animal welfare measurements are based on physiological responses, but most of these techniques are either invasive, expensive, time consuming or can cause study bias in response to an aversive stimulus during sampling (e.g. a blood draw)(Hill & Broom, 2009; Siegford, 2013). Thus, researchers are seeking alternatives methods that can evaluate animal well-being without compromising the animal they wish to evaluate. Some examples of these methods are the measurement of stress levels (Mormède et al., 2007), thermography (Nääs, Garcia, & Caldara, 2014), telomere attrition (Bateson, 2016), accelerometers (Whitham & Miller, 2016), among others.

Frequently, the welfare of animals is quantified, by investigating behavioural patterns that indicate problems (Dawkins, 2004) or stress hormone levels are investigated using non-invasive techniques, such as faecal or saliva sampling (Mormède et al., 2007; Möstl & Palme, 2002). Although these methodologies are

considered reliable (Hill & Broom, 2009; Walker, Díez-Léon, & Mason, 2014), there is still not a generalized methodology, which can be applied to any species of mammal and that does not require an experienced researcher conducting the study.

1.3 Sleep and Animal welfare

Measuring animal welfare can be challenging. This is because while some aversive stimuli are easy to identify and provide clear biological responses in terms of adaptation, such as food deprivation, others represent subjective changes in emotional states, which are difficult to assess (Broom, 1991; Mason & Mendl, 1993). Despite this, sleep is the only observable behaviour that indicates subtle changes in brain functions in direct response to the environment (Siegel, 2005).

Sleep has also been proven to be directly associated with quality of life in humans (the most investigated species in terms of wellbeing; Jean-Louis, Kripke, & Ancoli-Israel, 2000; Steptoe, O'Donnell, Marmot, & Wardle, 2008). Moreover, a literature review conducted by Langford & Cockram (2010) showed that the sleep/wake cycle in humans is not only highly dependable on physiological experiences that occur when awake, but also, the sleep disturbances that follow these experiences have a significant emotional component and directly reflect the subject's feelings.

1.4 The structure of sleep

Sleep is characterized as a momentary and reversible state of immobility, where the sensory responses are reduced (Cirelli & Tononi, 2008; Vassalli & Dijk, 2009). Despite several animal classes presenting similar behaviours to sleep, this activity is most evident in birds and mammals (S. S. Campbell & Tobler, 1984; Rial et al., 2010; Siegel, 2008).

In mammals, it is possible to identified two distinct stages of sleep: REM sleep (Rapid Eyes Movement) also known as paradoxical sleep and non-REM or slow wave sleep (Siegel, 2005; Harold Zepelin, Siegel, & Tobler, 2005). REM sleep is characterized by periods of brain activities generating spontaneous motor reactions. During this phase, the brain is as active as when is awake. It is also during this phase that our brain functions are restored and important processes, such as

the consolidation of memories, occur. Non-REM phase is identified by inactivity of the brain, with low emissions of brain waves, which reduces energy expenditure (Aulsebrook, Jones, Rattenborg, Roth, & Lesku, 2016; Elgar, Pagel, & Harvey, 1988; Hendricks & Morrison, 1981; Vassalli & Dijk, 2009). Likewise, this phase is responsible for the maintenance of bodily functions such as metabolism and immune response (Vassalli & Dijk, 2009; Harold Zepelin & Rechtschaffen, 1974).

Sleep cycles are highly polymorphic among mammal species; nonetheless, in all mammal species sleep cycles contain at least one alternation of non-REM to REM sleep or vice-versa (Tobler, 1995; Zepelin, Siegel, & Tobler, 2005). Humans, apes and other primate species often have a mono or biphasic sleeping rhythm, that is sleeping in one bout, while other species, such as ruminants, have polyphasic sleep (i.e., many phases of sleep and wakefulness in a 24 hour period; Elgar et al., 1988; Irene Tobler, 1995; Zepelin et al., 2005).

The duration of sleep cycles is species-specifics,(Figure 1) and It seems to be connected to several aspects of the species' biology and the environment. Such as, but not limited to: foraging, time spending eating, vulnerability to predators, availability of food resources, social structure, seasonal variations of the environment, body mass, and reproductive systems (Allison & Cicchetti, 1976; Elgar, Pagel, & Harvey, 1990; Horne, 2009; Phillips, Robinson, Kedziora, & Abeysuriya, 2010; Siegel, 2005).

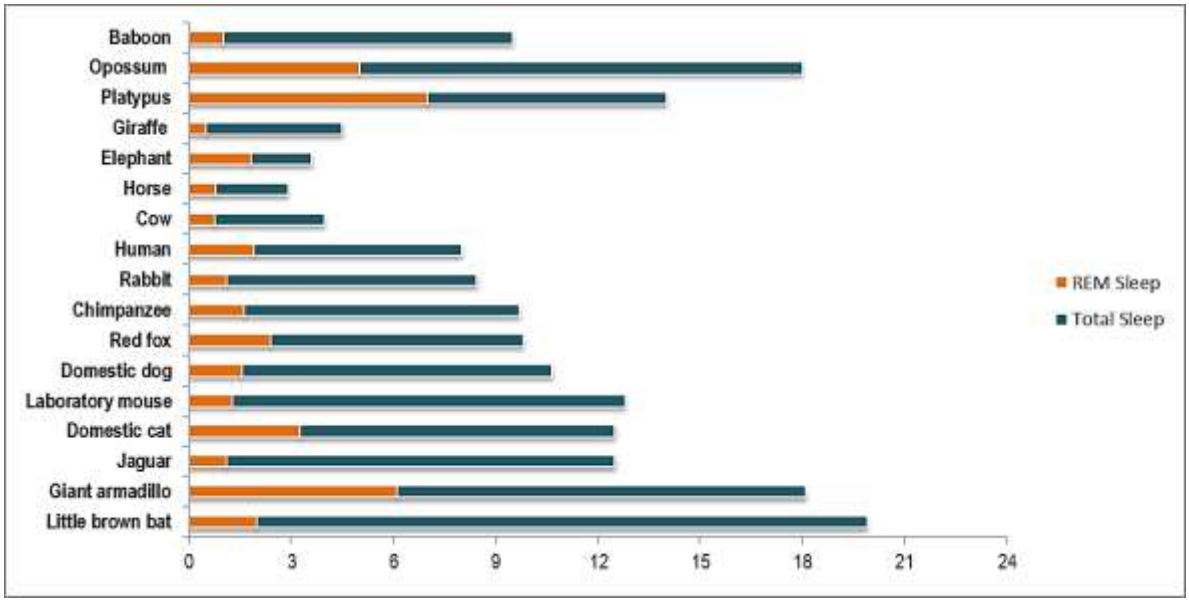

Figure 1. Comparison of total sleep duration in hours and REM sleep length between different terrestrial mammals. Data compiled from Zepelin, Siegel, & Tobler (2005) and Lesku, Roth, Rattenborg, Amlaner, & Lima (2008).

1.5 Sleep function and sleep deprivation

From parasite resistance to ensuring adaptive fitness several studies have debated the functions of mammalian sleep (Meddis, 1975; Nicolau, Akaârir, Gamundí, González, & Rial, 2000; Phillips et al., 2010; Siegel, 2005). It is theorized that sleep has the same motivation and functions across mammalian species (Siegel, 2005) and is a vital function (Cirelli & Tononi, 2008).

Sleep deprivation compromises several biological systems such as thermoregulation, energy balance, and immune function (Banks & Dinges, 2007; Knutson, Spiegel, Penev, & Van Cauter, 2007a; Okun, 2011).

The first reaction to sleep deprivation is the necessity of rebound sleep, which usually lasts longer than the normal sleep period (Orzeł-Gryglewska, 2010). However, when events of insomnia are recurrent, lack of sleep causes several physiological failures, which in extreme cases can lead to death (Cirelli & Tononi, 2008; Rechtschaffen, Bergmann, Gilliland, & Bauer, 1999).

An extensive review of the effects of sleep loss was conducted by Orzeł-Gryglewska (2010), and diseases such as diabetes, high blood pressure, obesity,

and even cancer are associated with the lack of sleep. Similarly, sleep loss is responsible for psychological problems such as depression, anxiety, and compromises the professional and personal performance of humans, impairing their cognitive abilities and causing memory loss (Capellini, McNamara, Preston, Nunn, & Barton, 2009; Drummond, 2001).

1.6 Sleep and stress

In humans, stress is the main cause of sleep disruption (Jun & Polotsky, 2016). Studies have shown that the hypothalamic-pituitary-adrenal (HPA) axis, responsible for the production of cortisol (a stress response product), which can affect sleeping patterns (Buckley & Schatzberg, 2005; Jun & Polotsky, 2016). Moreover, adrenal activity and production of cortisol is also regulated by a circadian rhythm, and similar to the sleeping/waking cycle, its production is modulated by light.

Cortisol secretion also exists in a direct and an inverted relation with the production of melatonin (sleep hormone) by the pineal gland (Buckley & Schatzberg, 2005). Cortisol is produced during the night and production peaks in the morning, whereas melatonin is produced late in the day inducing sleep, and continuously decreases during the night allowing cortisol to be produced (Buckley & Schatzberg, 2005; Monteleone, Fuschino, Nolfe, & Maj, 1992; Selmaoui & Touitou, 2003, Figure 2).

Chronic stress overstimulates adrenal activity increasing the production of cortisol, which then suppresses the production of melatonin, causing sleep fragmentation, which triggers more stress responses and consequently cortisol production, in a vicious cycle (Buckley & Schatzberg, 2005; Monteleone et al., 1992).

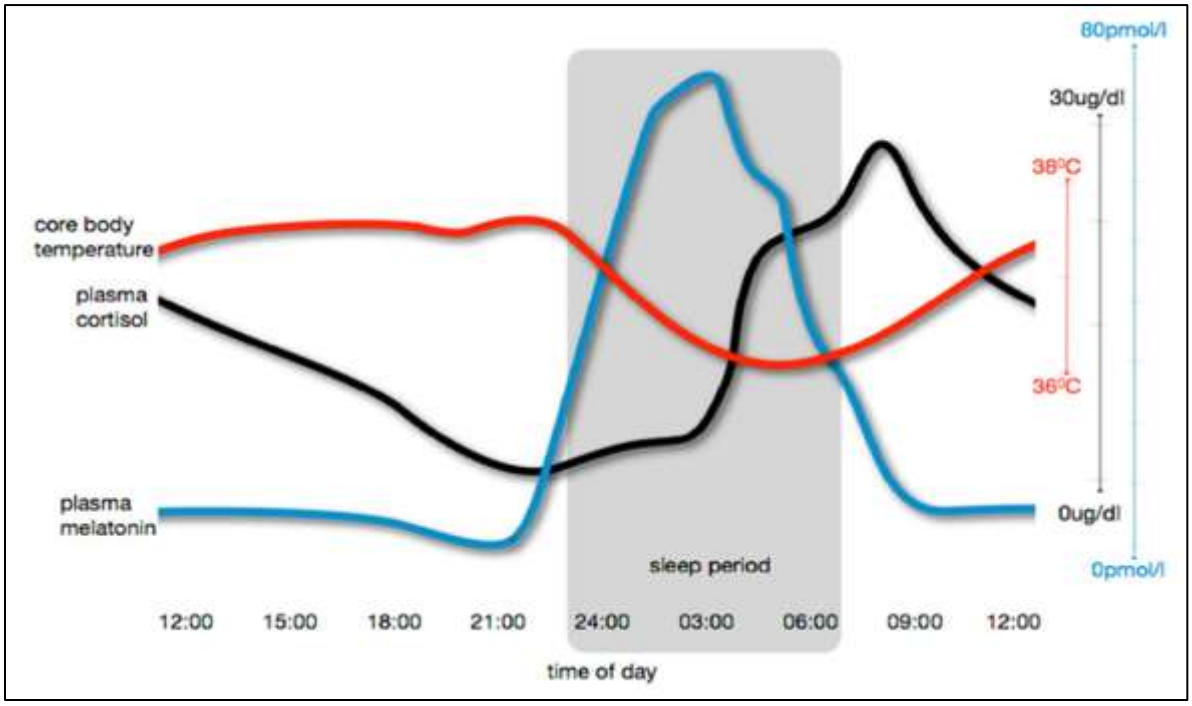

Figure 2. The normal synchronous relationships between sleep and day time activity and varying levels of cortisol, melatonin and body temperature (Hickie, Naismith, Robillard, Scott, & Hermens, 2013).

Stress affects sleep quality and quantity, reduces REM sleep, compromises the individual's immune systems, and increases the susceptibility to obesity and hypertension. It can increase aggression, induce anxiety, depression, impairs cognition and disrupts the circadian clock in both human and non-human mammals (Abou-Ismail et al., 2007; Hicks, Moore, Hayes, Phillips, & Hawkins, 1979; Kovalzon & Tsibulsky, 1984; Maggio et al., 2013; Pawlyk et al., 2008; Penev, 2007; Sadeh et al., 2004; Tobaldini et al., 2016; Vandekerckhove & Cluydts, 2010).

1.7 Domestic dogs as a model species to assess sleep welfare

Dogs have a long history of being a topic of interest in scientific and medical research (e.g Read, 1908) . As far as the documentation for modern medicine goes, multiples studies can be found using dogs as a comparative model for humans, within the most varied areas ranging from basic assessments of physiology and diseases to cancer and genomics research (Feuerbacher & Wynne, 2011; Fleischer, Sharkey, Mealey, Ostrander, & Martinez, 2008; Khanna et al., 2006; Tsai, Clark, & Murphy, 2007). Moreover, due to the unique relationship developed

through coevolution with humans, dogs present certain cognitive and behavioural traits that enable them to have similar responses to the environment and towards other individuals much more like humans than any other existent species (Udell & Wynne, 2008). Hence, they also make useful models when studying complex subjects such as brain development, cognition, evolution of social behaviour, and sleep disorders (e.g. narcolepsy) (Feuerbacher & Wynne, 2011; Kubinyi, 2006; Toth & Bhargava, 2013).

In recent years, the study of dogs as an interesting research species rather than a model also surfaced in several fields (e.g., veterinary, zoology, ethology, psychology) (Miklósi, 2016). Moreover, several characteristics allow the domestic dog to be a useful model species for animal welfare studies. First, they have well known physiology and behaviour, not only because of research, but because of veterinary care developed for pet dogs. Second, dogs are accessible in large sample sizes and easily trained (see review Arden, Bensky, & Adams, 2016). Third, pet dogs coexist with humans, which means they can provide information on how they cope with a world designed for humans (Ostrander, Galibert, & Patterson, 2000; Tsai et al., 2007; Udell, Dorey, & Wynne, 2010).

1.7.1 Dog sleeping patterns

Domestic dogs are a diurnal species (most behavioural processes happening during the day) (Miyazaki et al., 2002; Zanghi, 2010). Moreover, wakefulness comprises 70% of daytime in comparison to less than 40% in the dark cycle, where sleep related behaviours are predominant (Kis et al., 2014a; Miyazaki et al., 2002; Takeuchi & Harada, 2002; Brian M. Zanghi, 2010a). Nonetheless, dogs are also polyphasic sleepers, which mean they frequently transition in an out of sleep multiple times per night, and exhibit sleep (nap) bouts during the day (Kis et al., 2014b; Miyazaki et al., 2002; Takeuchi & Harada, 2002).

As humans, dogs sleeping cycles alternate between Slow-Wave sleep (NREM) stages and REM sleep, which comprises 20-36% of their sleep, occurs usually twice during a sleep cycle and is the phase with longer duration (± 83 minutes) (Kis et al., 2014a; Miyazaki et al., 2002; Takeuchi & Harada, 2002).

In contrast to humans, dogs' NREM are relatively shorter, while humans spent between 90 to 120 minutes in this stage (Carskadon & Dement, 2011), dog non-REM phases range between 15-30 minutes. In addition to shorter NREM cycles, dogs' express wakefulness during the sleep cycle in a proportion eight to 10 times greater than humans, and usually the awakening follows the end of a REM cycle (Kis et al., 2014a; Miyazaki et al., 2002; Takeuchi & Harada, 2002; Brian M. Zanghi, 2010a).

The sleep of dogs can be affected by several components of the environment such as feeding times (Zanghi, Kerr, de Rivera, Araujo, & Milgram, 2012; Brian M. Zanghi et al., 2013), activity (Bunford et al., 2018; Tobler & Sigg, 1986) and even affective states (Kis et al., 2017). Furthermore, biological factors such as age and sex also affect their sleep (Iotchev, Kis, Bódizs, van Luijtelaar, & Kubinyi, 2017; Iotchev et al., 2019; Zanghi, Kerr, DeRivera, Araujo, & Milgram, 2010). Ageing dogs present shorter sleep duration, more day time naps, less overall activity and more fragmented sleep compared to younger dogs (Part et al., 2014a; Siwak et al., 2003; Takeuchi & Harada, 2002), in the same way that ageing in humans causes chronic problems associated with disruptive activity/sleeping cycles (Carskadon & Dement, 2011).

1.8 Why investigate sleep and animal welfare

In studies of the most investigated species on the planet – humans – sleep quality is universally regard as an excellent measure of wellbeing, especially as it is so strongly associated with physical and psychological health (James, Honn, Gaddameedhi, & Van Dongen, 2017a; Schwartz & Roth, 2008; Vandekerckhove & Cluydts, 2010). Despite this, sleep has only recently drawn the attention of animal welfare scientists (Langford & Cockram, 2010). Given its universal importance across mammals in terms of their biological functioning and the fact that virtually all mammals (except, some marine mammals; Madan & Jha, 2012) are immobile when sleeping then this makes it ideal for automated monitoring.

1.9 Thesis Structure

The following chapters will explain how we employed a multidisciplinary approach to evaluate sleep as a measure of animal welfare in domestic dogs.

The research has been divided in four main methodological approaches: First, using behavioural observations, we investigated the sleep structure of the kennelled dogs and how sleep patterns and sleep fragmentation affect dogs overall behaviour (Chapter 2 and 3). Second, we explored how environmental conditions can affect dog sleeping cycles and how stress hormones impact/are associated with sleep quantity and quality (Chapter 4); third applying automatic monitoring by wearable technology, we measured physiological parameters and overall activity and sleep of the individuals, and explored further relationships between the variables. Moreover, we tested the efficiency of such method against the human-observed metrics of behaviour (Chapter 5). Lastly, using Artificial Intelligence (A.I. involving convolutional neural networks) and Computer Vision, a system was developed to "observe" sleeping dogs and quantify dog sleeping behaviour from videos and its validity/accuracy was tested against the manually (human) scored observations (Chapter 6).

The current research was approved by the Science & Technology Research Ethics Panel of the University of Salford Manchester (STR1617- 80) and by the Comissão de Ética no Uso de Animais of the Universidade Federal de Ouro Preto, Minas Gerais – Brazil (Protocol 2017/04).

In order to verify and quantify sleep, behavioural observations were carried across eight months with dogs in a laboratory facility in Brazil. The decision of using dogs was due to the extensive body of research about their physiology and behaviour, which makes them a good model for animal welfare. Moreover, the kennels were the dogs are kept are a constant environment with little interference of humans, meaning all the dogs live under the same circumstances and are affected by the same variables. The verified sleeping architecture of dogs is described in Chapter 2.

Using the metrics acquired during the data collection of sleep and behaviour, the duration of sleep and the number of sleeping bouts per night and per day was tested against the other categories of behaviour measured to further understand the impacts that sleep and sleep loss can have on the activity budgets of dogs (Chapter 3).

As the environment can affect sleeping patterns in dogs, we examined how components of the kennels such as light, temperature and sound could be interfering in sleep duration and sleep fragmentation (Chapter 4). Moreover, as stress responses are directly related to an individual's attempts to cope with its environment (B. Beerda, Schilder, Van Hooff, De Vries, & Mol, 2000), and as sleep is directly impacted by stress responses (Jun & Polotsky, 2016); cortisol levels from individuals were obtained from faecal samples during the same period of time that behaviour was being video-recorded. Further investigations explored the relationship between these measured variables (Chapter 4).

Sleep as an important part of the homeostatic process has a direct relation to other physiological measures (Vyazovskiy, 2015). In search of non-invasive methods to access such variables, as well to try and relate those variables to sleep, dogs wore a wearable sensor mounted in a collar (PetPace collar), which retrieved information about activity using an accelerometer and physiological measures such as heart rate and respiratory rate using an acoustic sensor. The variables measured by the collar were compared among themselves to verify how they could affect or be affected by sleep. In addition, the outputs from the collar were compared against the manually scored behaviours from the video to verify its accuracy (Chapter 5).

Finally, despite video-based observations being a tool to non-invasively monitor animal behaviour, the analysis of the results is very time consuming and prone to error. A practical solution to mitigate these problems is explored in Chapter 6. Using over 4000 hours of video acquired during the monitoring of the dogs, a system was developed in partnership with the University of Haifa in Israel, which combines deep leaning techniques (convolutional neural networks) with classical data processing methods to automatically detect and quantify dogs' sleeping patterns. The accuracy of the system was also tested against the manually scored sleep behaviour from the videos (Chapter 6).

Through these methodologies, the objective of this research is to verify if sleep has the potential to be used in animal welfare assessments, while also exploring the possibility of using new technologies to do so.

Chapter 2. Basic characteristics of sleep-related behaviours of kennelled laboratory dogs

2.1 Introduction

Sleep is a physiological process that is present in most animals (S. S. Campbell & Tobler, 1984; Siegel, 2008). The comprehensive study of the sleep/wake cycle through the decades with both human and several non-human models led to the conclusion that sleep is not a simple resting state, but an important part of the homeostatic process (Siegel, 2005; Vassalli & Dijk, 2009).

Sleep can be identified by three conditions: presence of a specific posture, a reduced but reversible responsiveness and homeostatic regulation (Vyazovskiy & Tobler, 2012). In mammals , it is normally divided in two different phases: REM sleep and Non-REM sleep, and each of the phases is connected with the restoration of different physiological and cognitive functions (Vassalli & Dijk, 2009; Vyazovskiy & Delogu, 2014; Vyazovskiy & Tobler, 2012).

Non-REM sleep is associated with the restoration of energy homeostasis, control of metabolic functions and immune systems, while REM sleep is connected to neuronal plasticity, memory consolidation and processing emotional information (Vassalli & Dijk, 2009; Vyazovskiy, 2015; Vyazovskiy & Delogu, 2014). Although not all the functions of the sleeping process are known, its adaptive value is undeniably high, since any changes in the sleep structure, no matter how small, will have deleterious effects on bodily functions (Banks & Dinges, 2007).

Sleep loss can be achieved by total or partial restriction of sleep or by fragmentation of sleep, which may or may not affect total sleeping times, but still has negative consequences (Meerlo, Sgoifo, & Suchecki, 2008). The lack of sleep has been associated with increased blood pressure, chemical imbalance, metabolic confusion, impairment of cognitive and locomotor functions. It can lead to extreme changes in behaviour, mental states and has even been associated with Parkinson's and Alzheimer's disease (Banks & Dinges, 2007; Hudson, Van Dongen, & Honn, 2019; Knutson, Spiegel, Penev, & Van Cauter, 2007b; Meerlo et al., 2008; Orzeł-Gryglewska, 2010).

In mammals, sleep is a species-dependent process, meaning its structure and timing are defined by ecological variables linked to environmental cues such as period of activity, feeding and reproductive cycles (Berger, 2011; Lesku, Roth II, Amlaner, & Lima, 2006; Savage & West, 2007). For instance, humans are monophasic sleepers, meaning their greatest period of sleep is concentrated in one single block over the dark phase of the day, whereas other species, such as dogs, are polyphasic sleepers and will have multiple bouts of sleep during a 24-hour period (Phillips et al., 2010). In addition, some species will have different sleeping postures which indicate the sleep phase they are in (e.g. lateral recumbence for REM-sleep in elephants) (Hediger, 1980; Williams, Bremner-Harrison, Harvey, Evison, & Yon, 2015).

Therefore, understanding sleep characteristics and especially sleep changes is relevant to the health and well-being of animals under human care. Despite several studies analysing the importance of sleep for animals and the extensive literature on humans and non-human animals such as mice and rats (Toth & Bhargava, 2013), only an initial understanding of all the variables that account for sleep in dogs is known (Zanghi, 2010)

Dogs have a diurnal circadian rhythm, and their activity peak occurs during the light period, whereas rest is most observed during the night (Miyazaki et al., 2002; Zanghi, 2010). The amount of time dogs spend sleeping is between 43-60% of a 24 hour period and REM-sleep occurs at rates between 20-36% of the sleeping period (Copley, 1976; Kis et al., 2014b; Takeuchi & Harada, 2002). Moreover, sleep in dogs varies with different biological characteristics, such as sex, age, levels of physical activity, feeding regimes, characteristics of the environment (e.g., presence of light) and even social interactions (Adams & Johnson, 1994a; Bunford et al., 2018; Fukuzawa & Nakazato, 2015; Iotchev et al., 2019; Kis et al., 2017; Owczarczak-Garstecka & Burman, 2016; Brian M. Zanghi et al., 2013b).

Usually, changes in the structure of sleep will be caused by events experienced during the period of wakefulness (Langford & Cockram, 2010). In humans and in rats, sleep disruption is associated with stressful events. Sleep disruption can also be considered a stressor by itself, since the lack of sleep compromises the entire biological system by overloading adrenal function,

suppressing immunity and, consequently, can be responsible for depressive states The same response is found in laboratory rats (Novati et al., 2008), therefore, dogs with disrupted sleeping patterns may be demonstrating reduced animal welfare.

This chapter focused on investigating the dynamics of the sleep/wake cycle of a group of laboratory dogs housed in a Research Centre from the University Federal of Ouro Preto (Brazil) – and how factors such age, sex and individual preferences play a role in the patterns of sleep behaviour.

2.2 Aim

To investigate sleeping patterns of kennelled laboratory dogs and the biological factors that contribute to its structure.

2.3 Methods

2.3.1 Study location

This study was developed with a group of dogs belonging to the Centre of Animal Science of the Federal University of Ouro Preto, located in the city of Ouro Preto, state of Minas Gerais – Brazil (20 ° 23'41.7 "S 43 ° 30'39.6" W).

The Centre of Animal Science has three main kennels, two destined for research - Kennels A and B - and one kennel for breeding, Kennel C (also designated as Nursery). All dogs in our study belonged to Kennel C (Figure 3) and were not taking part in any other type of research nor being used for breeding during the entire duration of the data collection.

The area of Kennel C consists of 20 kennels divided in two different sections by dog's sex. All kennels have a basic rectangular layout of 5.8m x 1.6m x 1.65m with bare concrete walls and flooring. The kennels do not have insulation nor have beddings, toys or any other items aside from the dogs' food and water bowls. One-third of the kennel space is covered by a ceiling made of asbestos tiles, which provides shelter for the dogs. The female kennels also have a small room on the back that provides shelter and is used as a birthing den whenever the dogs are

breeding (Figure 4). In all the kennels, dogs had natural light (no artificial lighting was provided).

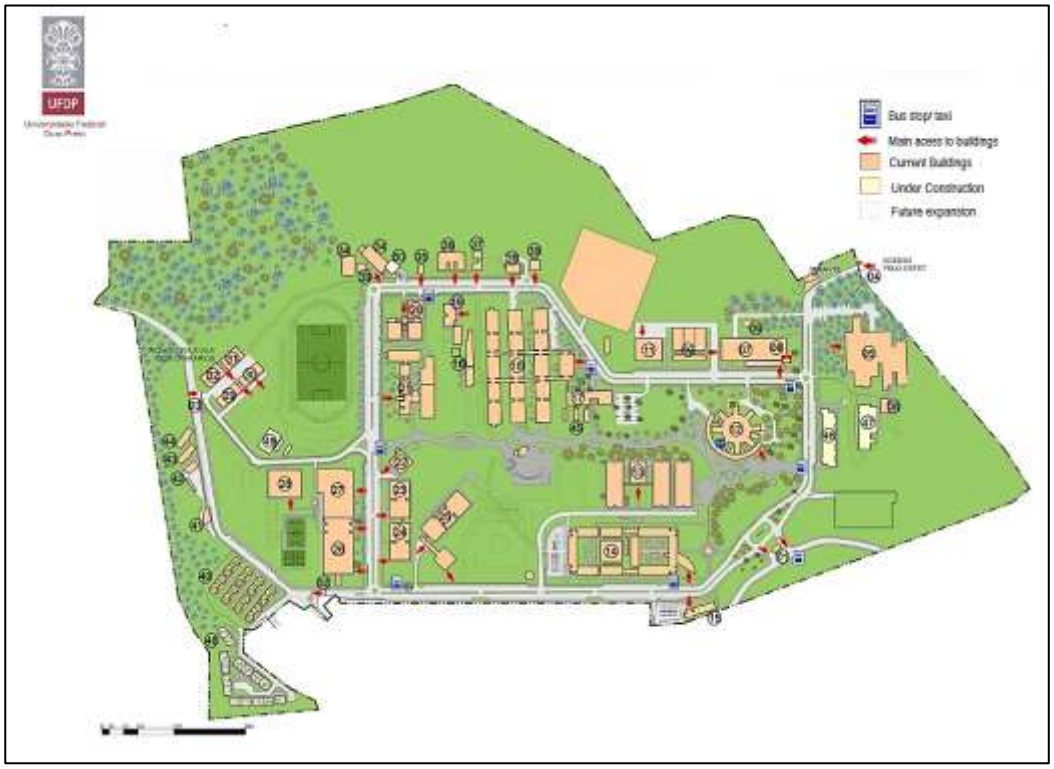

Figure 3. Map of the Federal University of Ouro Preto campus in Minas Gerais, Brazil. Number 36 indicates the Centre of Animal Science on the map. Number 37 indicates Kennel C (Nursery), Numbers 38 and 39 indicate research labs on the map.

Figure 4. Front view of kennels. Example of a kennel in the male wing (A). Example of a kennel in the female wing (B).

2.3.2 Subjects

For this study, 13 mix-breed adults' dogs, seven males (5.1 ± 1.7 years old) and six females (6.6 ± 1.9 years old) were randomly selected. All the dogs were kept in pairs, aside from one male, who was housed alone.

Dogs had access to water and food *ad libitum*, which were replaced if necessary, during cleaning routines. All the dogs were visually inspected (daily) by veterinary staff and received care whenever necessary. Dogs were not walked, nor received any type of enrichment, instead, once a week; dogs got a "play" session where all individuals from the same sex were released together for an hour to exercise. Contact with conspecifics of opposite sex was not allowed to prevent unnecessary breeding. At the beginning of the study, all animals were checked-up by the Chief veterinarian, who attested to them being in perfect health and behaving normally (i.e. no clinical signs of behaviour changes, such as limping, self-injury, among others). Dogs only encountered staff during inspections or cleaning, but physical contact was restricted to veterinarians.

Table 1. Descriptive characteristics of the 13 dogs participating in the study

ID	Sex	Age	Weight	Paired dog	Behaviour towards humans
Alfafa	Male	3	19.5 kg	Aquiles	Sociable
Amarelo	Male	7	24.9 kg	Amaro	Sociable
Amaro	Male	2	21.7 kg	Amarelo	Sociable
Apolo	Male	3	31.7 kg	Arisco	Fearful
Aquiles	Male	3	20.8 kg	Alfafa	Neutral
Arisco	Male	3	26.3 kg	Apolo	Fearful
Atila	Male	2	29.0 kg	-	Fearful
Bolinha Filha	Female	4	20.4 kg	Bolinha mae	Sociable
Bolinha Mae	Female	5	16.7 Kg	Bolinha filha	Sociable
Grace	Female	6	30.8 Kg	Tammy	Neutral
Meme Marrom	Female	7	16.7 Kg	Meme Preta	Neutral
Meme Preta	Female	7	18.1 Kg	Meme Marrom	Neutral
Tammy	Female	7	9.0 kg	Grace	Neutral

2.3.3 Data collection of behavioural data

In order to monitor the dog's sleep behaviour, eight CCTV cameras with night vision capability (Swann SWDVK-845504) were installed in four kennels, two in the female wing, and two in the male wing (each kennel had two cameras to ensure dogs were visible at all times) (Figure 5).

Figure 5.Monitor displaying the transmitted footage of the eight cameras installed in the kennels (A). Snapshot of nocturnal footage taken from a kennel on the male wing (B). Detail of the CCTV camera installed in the kennel (C).

From October 2017 to May 2018, 3120 hours of video, accounting for 130 days and nights were collected from the kennels. Each sample comprised of a continuous 24-hour, five-day assessment period, from Monday afternoon to Saturday morning.

For extraction of the behavioural data, each 24-hour period was further divided into two sampling periods: diurnal (07:00-17:59h) and nocturnal (18:00-06:59h). For the diurnal period, focal animal sampling with instantaneous recordings of behaviour at a fixed time interval was used (Martin & Bateson, 2007). Dogs were assessed each hour, for 15 minutes, at 30 seconds intervals, totalling 30 recordings per hour. The behaviours were classified using an ethogram (Table 2) based on scientific literature for dogs (Luescher et al., 1991; Broom & Fraser, 2015) and the data were scored with the assistance of the software Boris v.7.0.12 (*Behavioural Observation Research Interactive Software,* (Friard & Gamba, 2016).

In addition, if the dogs engaged in sleep behaviour during the day, the total time of sleep was recorded and if recurrent, the total number of sleeping bouts for that period was counted. For the nocturnal period, focal animal sampling with continuous recordings of behaviour was used (Martin & Bateson, 2007). Due to the large number of data that continuous observation generates, the behaviours observed at night were allocated into three broad categories: sleep, rest and activity. Additionally, we scored the number of sleeping bouts per night, the latency until the first sleeping bout and the last bout before the diurnal observations period began. During all times the dogs were scored as being asleep (both during day and night) the position in which the animal slept was also recorded.

Table 2. Ethogram used to assess diurnal and nocturnal behaviours of dogs at the Centre of Animal Science, UFOP, Brazil.

Behavioural states*		Definition
Consumption	Feed	Dog is actively consuming food.
	Drink	Dog is actively consuming water.
Interaction	Explore	Dog interacts with the environment approaching surfaces in an investigative manner (smell, scratch, lick, touch, and dig).
Locomotion	Locomotion	Dog is moving from one location to another with alternate limbs touching the ground at different times throughout.
Rest	Resting	Dog is laying down with abdomen touching the ground with either dorsal, lateral, or caudal side and stationary. Limbs can be stretched in the front, laterally or curled near the body. Eyes open and movement of ears can be observed.
	Sleep	Dog has similar positions as resting, but eyes remain closed for at least 02 minutes. Paddling of limbs, rapid eye movement and occasional vocalizations can be expressed.
Stationary	Alert	Dog is in is in upright position with all limbs extended. Ears are in upright position. Ears and head are pointing into the direction of stimuli.
	Sitting inactive	Dog is in upright position, with hind legs flexed while front legs are extended straight.
	Stand inactive	Dog is in upright position with all limbs extended supporting the body while immobile.
Social	Social Affiliative	Dog is interacting (rub, smell other, present, mate or groom other) with a conspecific in an affiliative manner.
	Social Aggressive	Dog is interacting (bite, chase, strike with paw, show teeth, hair bristle) with a conspecific in an aggressive manner.
	Play	Dog engages with a conspecific, less than two body lengths away, and different behaviours are observed (jump over other, chase, pulling tail, mouth, roll).
Maintenance	Groom	Dog grooms itself using tongue, teeth or mouth.
	Excrete	Dog releases faeces or urine from the body.
	Tail-chasing	A quick and repetitive circular motion, where the dog attempts to bite its tail.

Repetitive**	Pacing	A locomotor movement with the animal traversing the same pathway at least twice in quick succession.
Panting**		Dog's mouth is open, tongue out. Dog quickly inhales and exhales through mouth breathing. Chest breathing movements are visible.
Vocalization**	Rhythmic Barking	Dog opens and closes mouth while emitting repetitively short and constant vocalizations that can vary in frequency from very high to deep and low.

* Based on Broom & Fraser (2015) **Based on Luescher, McKeown, & Halip(1991).

2.3.4 Statistical analysis

All behavioural data were tested for normality using the Anderson-Darling normality test. All statistical tests were considered significant at $p<0.05$. Descriptive statistics of all the analysed metrics were conducted and results are presented as either counts or percentages with standard deviation.

To verify the differences in the amount of time spent asleep and number of sleeping bouts during day and night, a Wilcoxon test was performed. This test was chosen because the samples were related (Dytham, 2011).

The relationship between the different categories of activity was verified using a Friedman test with Dunn's Post-hoc, in order to decide which groups were significantly different from each other (Dytham, 2011).

The correlation between sleep duration and sleep fragmentation was tested using a Polynomial Regression analysis. In a simple linear regression, the relation between the dependent (y) and independent variable (x) is explained by fitting the linear equation $y= a +bX$ and the residuals are normally distributed. Through a scatterplot was possible to identify that the residuals were not normally distributed, hence a Polynomial regression was chosen as the appropriate model to conduct the analysis. The polynomial regression assumes that the value of Y is modelled as an *n*th degree polynomial in x by fitting the equation $y = a + b_1X + b_2X^2 +\dots b_nX^n$. After transforming the data through the polynomial equation, the best-adjusted curve was found to be the one in a polynomial regression with quadratic transformation, where the variation of Y is explained by the variation of X^2 (Dytham, 2011).

The relationships between sleeping metrics, such as sleeping bouts or sleeping duration and other variables, such as sleeping positions, age, sex, and latency were analysed using multiple linear mixed effect models tests (GLMMs, lmer function for data with normal distribution and glmer for non-normal data, both with lme4 package in R; Crawley, 2007). Due to the metric difference of each of the variables included in the models (e.g. sleeping duration in seconds vs total count of behaviour) before carrying out the tests, the data were scaled to reduce size difference and avoid over dispersion of residuals. To scale the variables, the entire matrix mean and standard deviation is calculated, than each element in the matrix is scaled by subtracting the mean and dividing by the standard deviation (Becker, Chambers, & Wilks, 1988).

The reported results from the GLMM tests were selected through the elaboration of a minimal model based on the variables tested. The first model (M1) include all the explanatory variables against the intended response. From the M1 the least explanatory variable of that model (least non-significant in the ANOVA test) was excluded from the model and a new GLMM was carried (M2). Each model was tested against the previous model using an Analysis of Variance, to ensure the variable could be extracted from the model, which occurred when the ANOVA was not significant between models. The last model – the minimum model – was the one in which none of the variables could be further removed (i.e. the ANOVA test between the models was significant).

As the dogs were housed together, there was a possibility for pseudo replication of behaviour between paired dogs. To address this, the variable kennel was included in the models as a random effect in addition to date. However, preliminary results (Appendix 1) showed a problem with singularity (dispersion of residuals close to zero) when running the model including both Kennel and Date as random effects. Since the variation on daily sleeping habits was important to understand sleep quality in the tested dogs, a practical decision was made to remove the variable kennel from the analysis. Using only date as a random effect did not return a singularity problem, hence all further analysis in this and in further chapters considered only date as a random effect whenever necessary.

Finally, to understand if the fragmentation of sleep affected subsequent nights of sleep, we conducted a Ttme series analysis through a temporal autocorrelation, which correlates observations of a time series separated by n units of time. In our case, number of sleeping bouts per night by the number of days observed (Crawley, 2007).

All statistical analyses were carried out using Studio R (RStudio team, 2019).

2.4 Results

2.4.1 Overall sleep and activity profiles of dogs

From 1560 hours of video, descriptive statistics for measures of sleep and activity were calculated for each dog (Table 3). On average, the dogs slept 6.1 ± 3.9 hours (23777 ± 13932.4 seconds) with a mean of 10.8 ± 7.01 bouts per night. Over 65% of the time, the first sleeping bout occurred between 18:00 and 19:00 hours and the dogs woke up, on average, 20 minutes before the morning shift of the kennel started (at 07:00 hours). During the day, dogs were recorded sleeping only 6.02% (± 0.28) of the total observations with an average duration of 92 ± 391 seconds and with less than one bout per day (0.14 ± 0.43).

The amount of time sleeping was significantly different between day and night ($Z= 5.65$, $df= 1$, $p < 0.001$), as the number of sleeping bouts ($Z= 31.39$, $df = 1$, $p<0.001$), with the dogs sleeping more during the night. Conversely, the amount of time spent asleep, active or inactive during the night were also significantly different ($F= 177.5$, $p<0.001$, $df = 2$), with sleeping being the most expressed behaviour (Figure 6).

Although sleep was not observed frequently during the day; the amount of time spent inactive accounted for 61 % of total observations [“Sitting inactive” (13.56 % ± 4.89), “Standing inactive” (15.12% ± 4.43), “Lay down inactive” (32.64% ± 8.07)]. Inactivity was significantly different from sleep and activity and the most expressed diurnal behaviour ($F= 1844$, $p<0.0001$, $df= 2$, Figure 7).

Table 3. Individual characteristics of sleep found in the dogs participating in the study over 160 nights.

ID	Sleep (±SD)[1]	Sleep bouts (±SD)[2]	Duration of bouts[3]	Time spent resting (%)[4]	Time spent active (%)[5]
Alfafa	5.0±4.6	9.1±9.07	39'20	79.11	20.89
Amarelo	5.5±4.6	10.7±5.9	29'31	69.21	30.79
Amaro	6.2±3.8	10.1±5.8	35'22	70.59	29.41
Apolo	9.1±1.6	17.2±4.0	32'24	84.04	15.59
Aquiles	5.5±4.1	9.8±9	44'31	72.40	27.60
Arisco	8.9±1.6	15.7±4	36'14	80.59	19.05
Atila	6.1±3.9	12.1±7.8	30'06	75.49	24.51
Bolinha Filha	5.9±4.0	7.4±5.4	60'24	79.79	20.21
Bolinha Mae	7.1±4.2	8.1±5.9	66'55	82.34	17.66
Grace	7.1±3.3	13.5±6.6	31'07	77.38	22.62
Meme marrom	7.4±4.7	7.9±5.8	64'04	88.64	11.36
Meme Preta	7.2±4.6	8.1±5.9	61'13	90.99	9.01
Tammy	5.4±4.3	10.6±7	28'36	69.30	30.70

[1]Mean time expressed in hours. [2]Mean per observed night. [3] Total time spent asleep divided by number of bouts. Values express average in minutes. [4] Mean percentage of time spent asleep and inactive during the night.[5]Mean percentage of time spent awake during night.

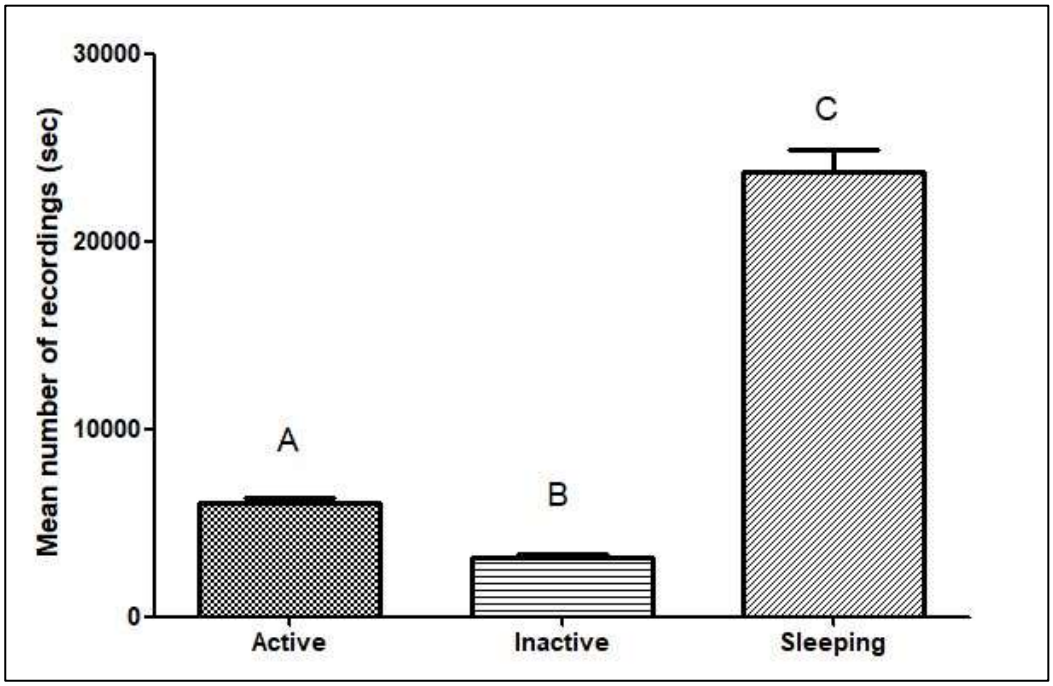

Figure 6. Mean expression of different behavioural categories during nocturnal observations of dogs over 160 nights. Capital letters show significant differences ($p<0.05$) between categories. Error bars show ± standard error of the mean.

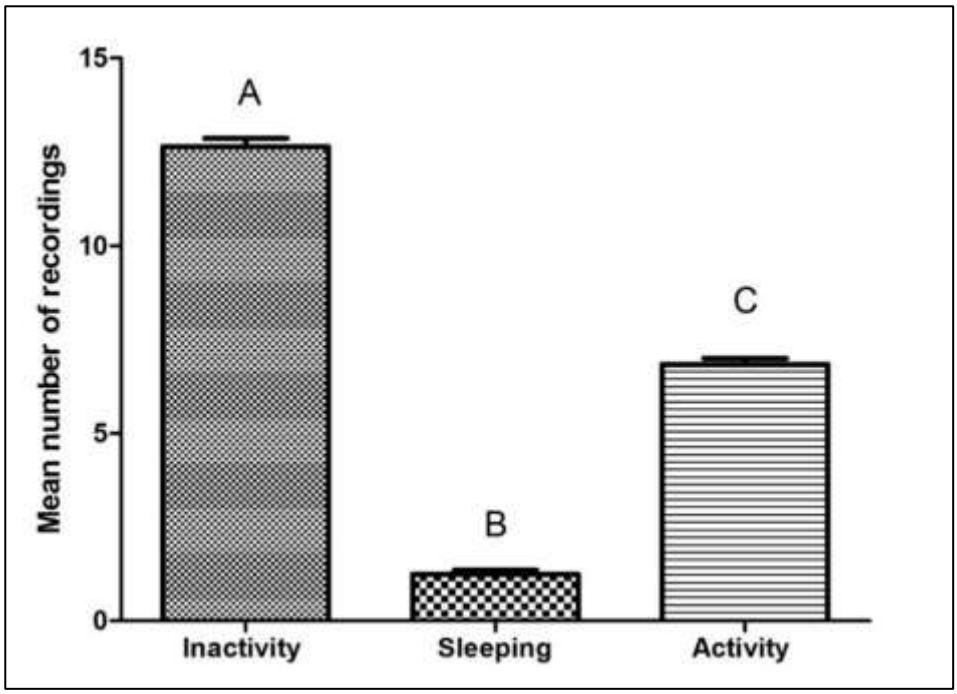

Figure 7. Mean expression of different behavioural categories during diurnal observations of dogs over 160 days. Capital letters show significant differences ($p<0.05$) between categories. Error bars show ± standard deviation of the mean.

Using General Linear Mixed Effect models, age and sex were tested in response to sleep duration and numbers of bouts, both at day and night. Significant effects ($p<0.05$) were found for both factors and results are summarized in Table 4.

Post-hoc tests indicated that males tended to wake up more during the night than females, while females slept more frequently during the day (Figure 8). Older dogs also slept more during the day (frequency and duration) and had more sleeping bouts than younger dogs (Figure 8). Sleep duration was not different between sexes during the night, nor was the number of sleeping bouts during the day.

Table 4. Linear Mixed Effects Models results for the optimal models describing the relationship between age, sex, and sleep metrics as a predictor variable for dogs.

Response	Parameters	Estimate ± SE	Coefficient value	p	Error distribution
Sleep bouts (night)	Intercept	11.3513 ± 0.831	1.144	ns	Gaussian
	Age	0.2815 ± 0.3118	0.903	ns	
	Sex [a]	4.5118 ± 1.4163	3.190	0.001**	
Sleep duration (night)	Intercept	13734.5± 9206.6	0.715	ns	Gaussian
	Age	27.9782± 582.04	0.048	ns	
	Sex [a]	2242.05± 2645.8	0.847	ns	
Sleep bouts (Day)	Intercept	-2.9472± 0.3271	-9.009	$2e^{-16}$***	Poisson
	Age	0.2033± 0.0453	4.493	$7.03e^{-16}$***	
	Sex [a]	-0.2331± 0.1917	-1.216	ns	
Sleep duration (day)	Intercept	-0.1955± 0.1307	-1.495	ns	Gaussian
	Age	0.0637± 0.0913	3.295	0.001**	
	Sex[a]	-0.0507± 0.0869	-0.583	ns	
Sleep Count (day)[b]	Intercept	-1.4220± 0.4323	-3.289	0.001**	Poisson
	Age	0.2334± 0.0167	13.979	$2e^{-16}$***	
	Sex [a]	-0.7909± 0.0719	-10.993	$2e^{-16}$***	

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. [a] Male is the reference group. [b] Number of recordings.

Further investigation of the differences between males and females were performed using Generalised Linear Mixed Models tests, where the subjects were used as explanatories variables and tested against the sleeping bouts and sleeping duration at night and the sleeping bouts and sleeping duration during the day.

The *post-hoc* tests showed that during the night, the male Apolo (t= 2.950, p <0.001, Family = Gaussian) woke significant more than all the other dogs (Figure 9). During the day, the variation increased with several dogs sleeping more than others, but the females Tammy (t= 7.277, p< 0.001, Family = Gaussian) and Grace (t= 3.096, p<0.001, Family = Gaussian) are the dogs who slept significantly more (Figure 10). Similarly, the number of bouts during the day was also higher for these females [Tammy (z= 3.025, p<0.01, Family = Poisson), Grace (z= 1.791, p<0.05,

Family = Poisson)], but other dogs also presented significant variations (Figure 11). Sleeping duration at night was not different among dogs.

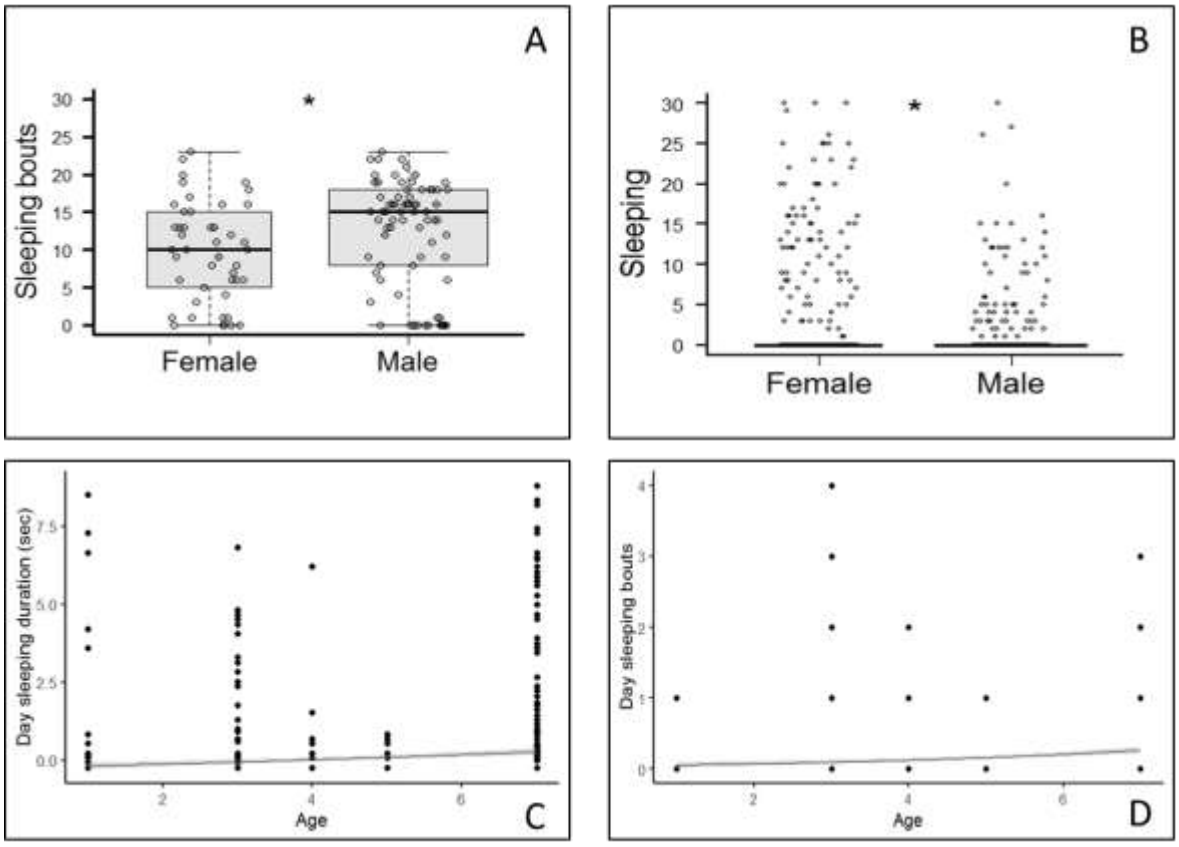

Figure 8. Age and sex patterns of sleep metrics. A) Number of nocturnal sleeping bouts for female and male dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. * Indicates significant difference at $p<0.05$. B) Day sleep recordings for female and male dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges * Indicates significant difference at $p<0.05$. C) Relationship between duration of sleep during the day and age. Trend line based on GLMM result: -0.1955+ 0.0637*Age. D) Relationship between number of sleeping bouts during the day and age. Trend line based on GLMM result: -2.9472+ 0.2033*Age.

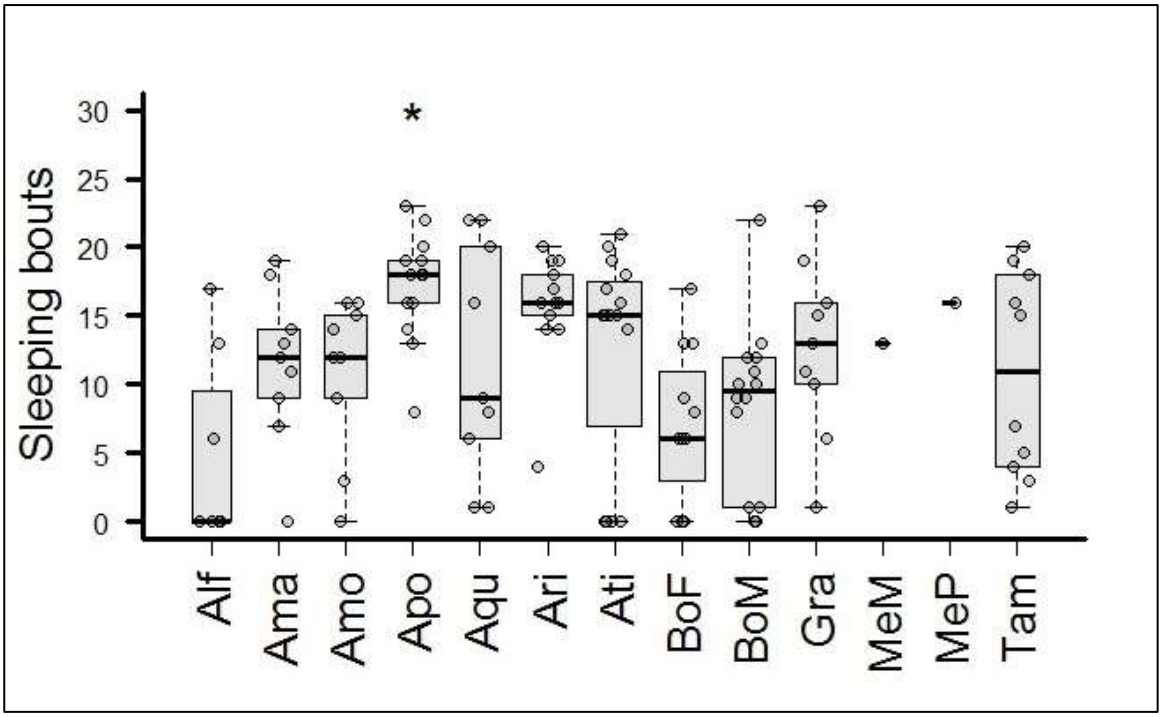

Figure 9. Individual variation of sleeping bouts recorded during nocturnal observations for the observed dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. * Indicates significant difference at p<0.05.

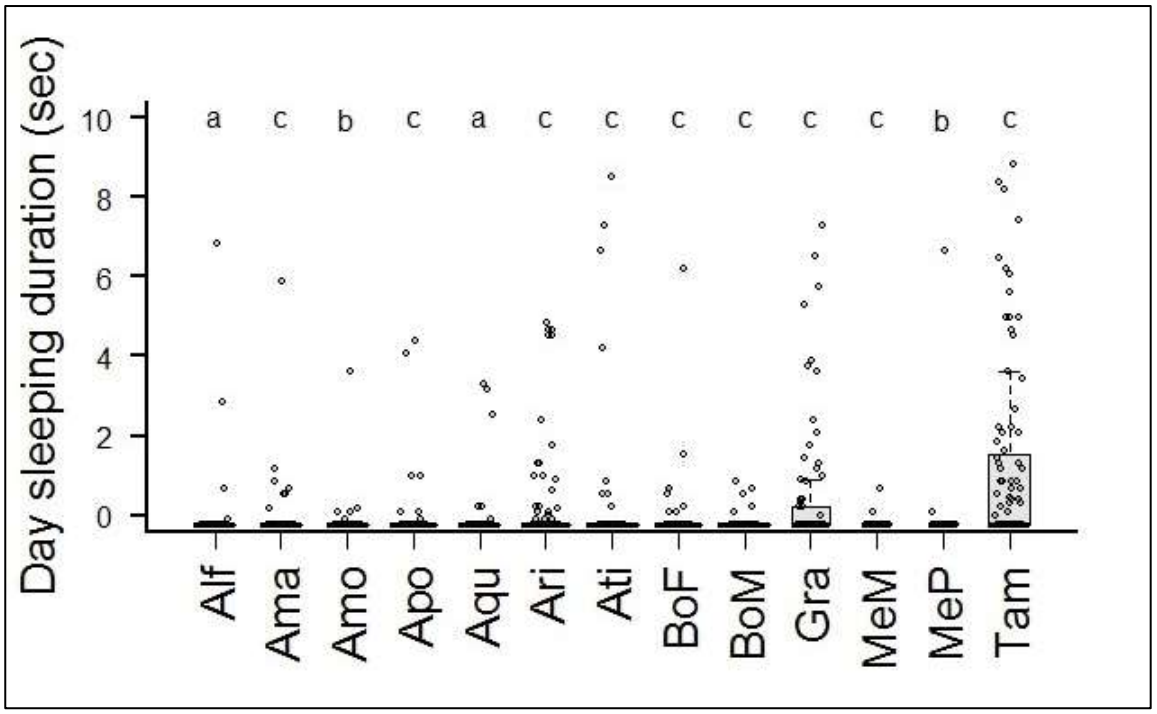

Figure 10. Time spent sleep recorded during the day for individual dogs (Sleep was scored after two minutes of continuous inactivity with eyes closed). Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. Lower case letters indicate significant variance at p<0.05.

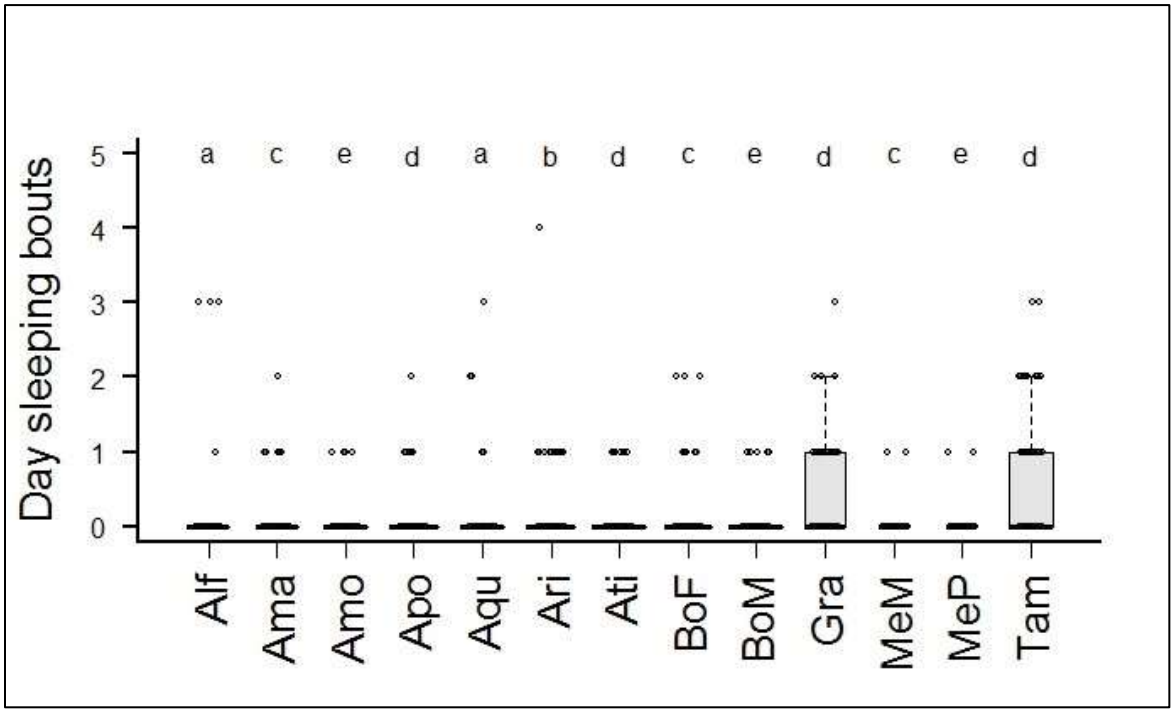

Figure 11. Individual variation of sleeping bouts recorded during the day for the observed dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. Lower case letter indicates significant variance at $p<0.05$.

A polynomial regression analysis (Table 5, Figure 12) identified that sleep fragmentation (sleeping bouts) caused an increase in the percentage of time spent asleep during the night ($r^2= 0.66$, $F= 140$, $df= 144$).

Table 5. Polynomial regression with quadratic transformation results for the effect of sleeping bouts on sleep duration at night.

Parameters	Polynomial regression coefficient (±SE)	t value	Pr(>|t|)
Intercept	19.2815± 3.3517	5.753	$4.92e^{-08}$***
X	9.2729± 0.6732	13.774	$2e^{-16}$***
X Sq.	- 0.3138± 0.0302	- 10.373	$2e^{-16}$***

*** $p \leq 0.001$

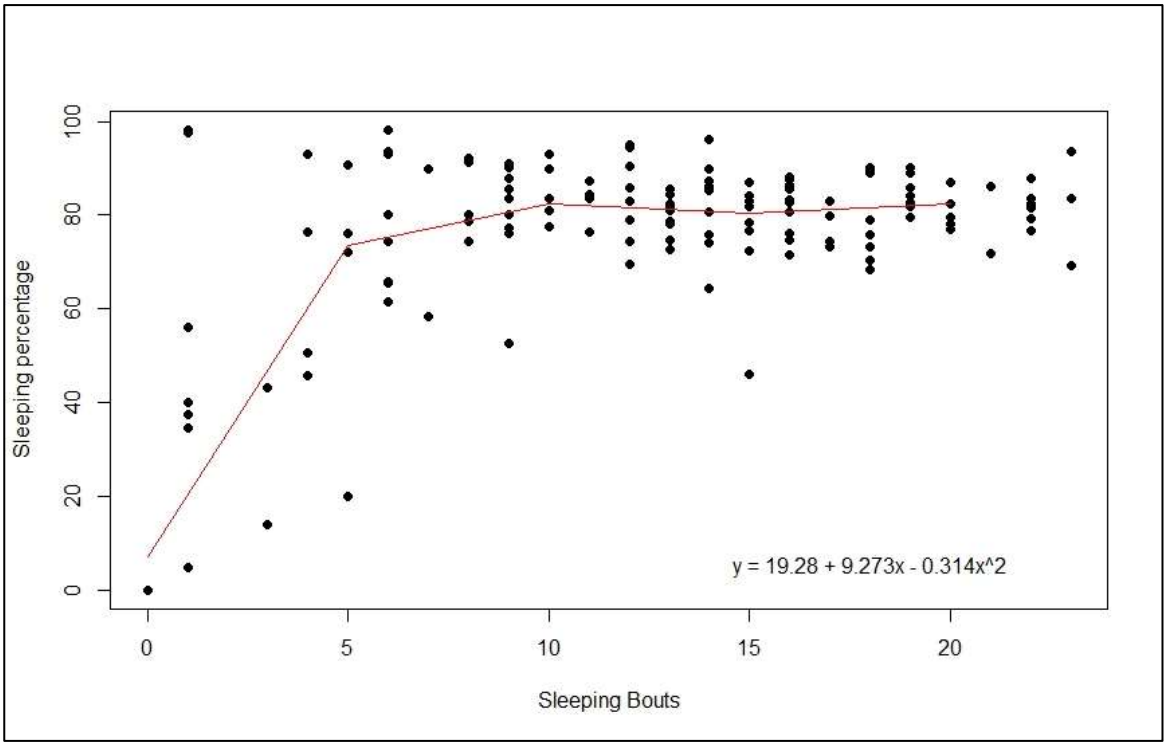

Figure 12. Sleeping percentage variation in response to the number of sleeping bouts. Trend line based on polynomial regression result: Percentage of sleep = 19.28 + 9.273(bouts) – 0.314(bouts)^2, r^2= 0.66.

Moreover, a Generalised Linear Mixed Model analysis indicated that sleep duration in a previous night had influence on the sleep duration on the following nights (F= 4.3409, df=34, p <0.001). However, the *post-hoc* test did not establish a pattern, with only some specific nights affecting the subsequent night. Thus, a temporal autocorrelation was performed to try to identify if the individuals' sleeping patterns would affect the temporal distribution of sleep.

The results of the temporal autocorrelation showed that the total amount of time spent asleep did not influence sleep duration in following nights for the dogs (p>0.05). Nonetheless, when we used the number of sleeping bouts instead of the sleeping duration, it was possible to find an effect on the amount of sleep (p<0.05).

Two dogs showed a significant relation between the amount of sleep in the first night and some subsequent night. Aquiles' (male) sleeping pattern showed that the amount of sleeping bouts in the first night affected negatively the time spent asleep and five nights later, it would sleep less (Figure 13). In addition, Tammy (female) showed that the number of bouts in the first night would positively affect

the second night, with the subject sleeping more in that night than in the following nights (Figure 14).

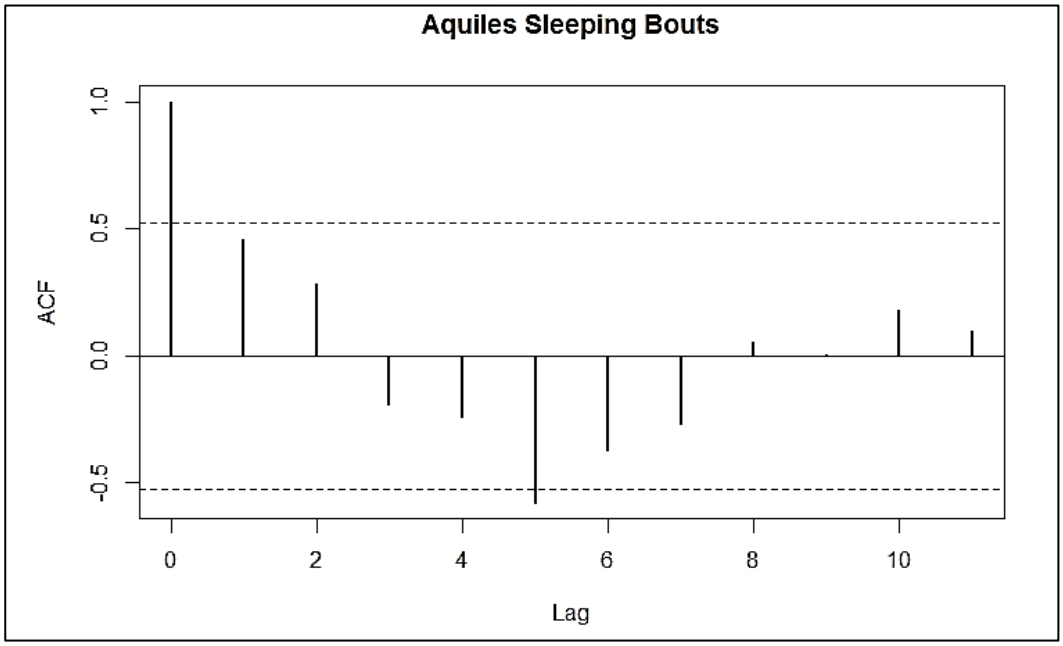

Figure 13. Time series analysis showing the relationship between the number of sleeping bouts and subsequent amount of sleep. Bar above/below dotted lines express significant autocorrelation ($p<0.05$). Lag = Number of nights. ACF= Auto correlation factor.

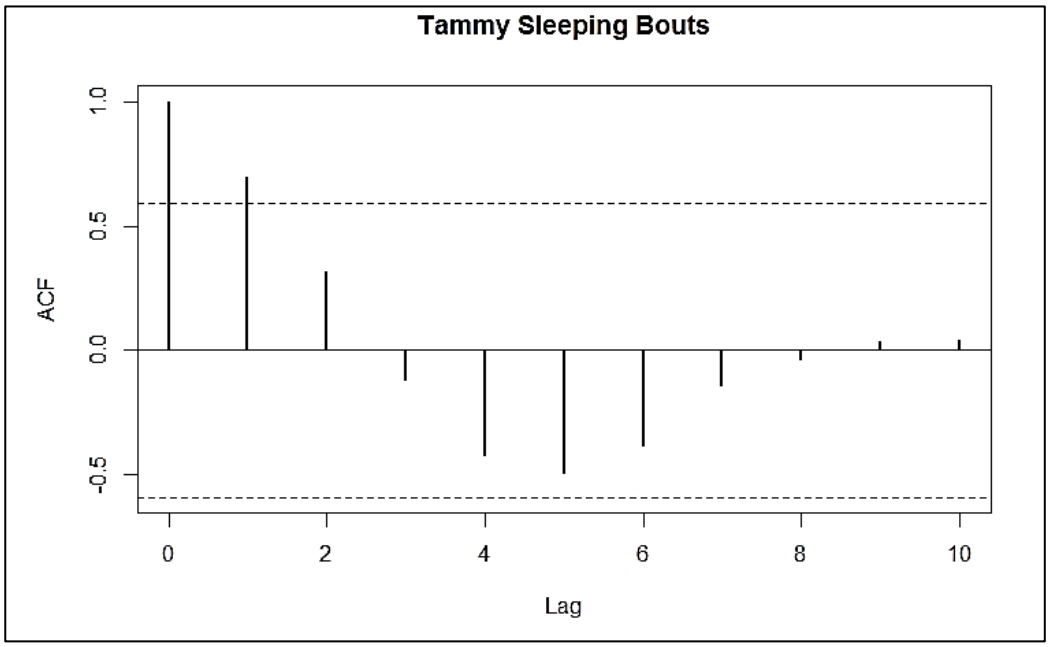

Figure 14.Time series analysis showing the relationship between the number of sleeping bouts and subsequent amount of sleep. Bar above/below dotted lines express significant autocorrelation ($p<0.05$). Lag = Number of nights. ACF = Auto correlation factor.

2.4.2 Sleep positions

While measuring sleep on the dogs, it was possible to observe a variation during the night on the positions adopted by each dog to sleep. The amount of time spent sleeping in each position was significantly different ($F = 260$, $p<0.001$, $df = 3$), with dogs preferring to sleep either on their left or right flank than on their sternum or their back (Figure 15).

Results from the GLMM models showed that the time spent in each position was also affected by the number of bouts, with dogs spending more time sleeping on their right side ($t= 9.086$, $p<0.001$, family = Gaussian) or sleeping on their left side ($t= 5.823$, $p<0.001$, family = Gaussian) with the increase of sleeping bouts.

Males and females did not differ on the time spent asleep in each position; on the contrary, age did influence choosing to sleep on the back position, with older dogs sleeping less in this position than younger ones ($t= -1.933$, $p<0.05$, family = Gaussian). When assessing individuals' sleeping positions, the models pointed to variations on sleeping right, sleeping sternally and sleeping on back ($p<0.05$). The post-hoc test showed that the male Apolo had a stronger preference for sleeping in his right side (Figure 16), the male Aquiles to sleep in his chest (Figure 17), and Atila was the subject with most recordings for sleeping on his back (Table 6).

Table 6. GLMM results for the optimal models describing how dog sleep positions varied with sleeping bouts, age, sex and individuals.

Response	Parameters	Estimate ± SE	t value	p
Sleeping right	Sleeping bouts	0.0988 ± 0.0108	1.144	0.001
	SubjectApolo	1.9118 ± 0.4028	4.745	0.028
Sleeping left	Sleeping bouts	0.0696 ± 0.0119	5.823	$5.76e^{-09}$
Sleeping sternally	SubjectAquiles	2.4951± 0.4333	5.758	0.050
Sleeping back	Age	-0.1206 ± 0.0624	-1.933	0.053
	SubjectAtila	1.3314 ± 0.4719	2.821	0.004

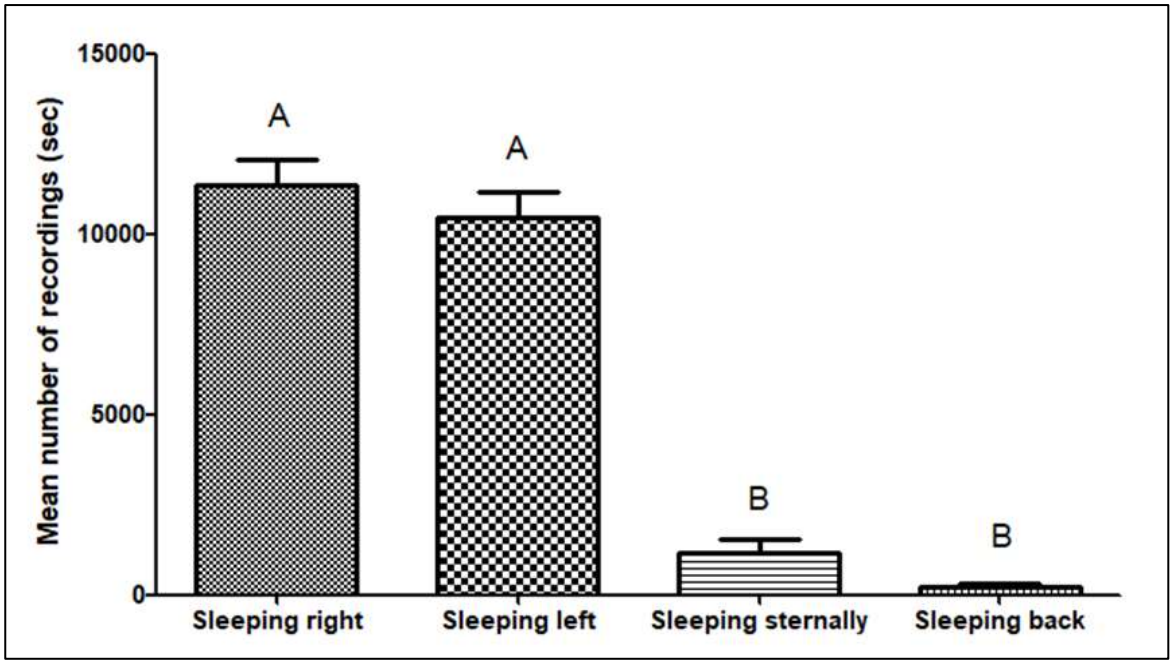

Figure 15. Mean values of sleeping positions recorded for dogs over 160 nights. Capital letters show significant differences ($p<0.05$) between categories. Error bars show ± standard error of the mean.

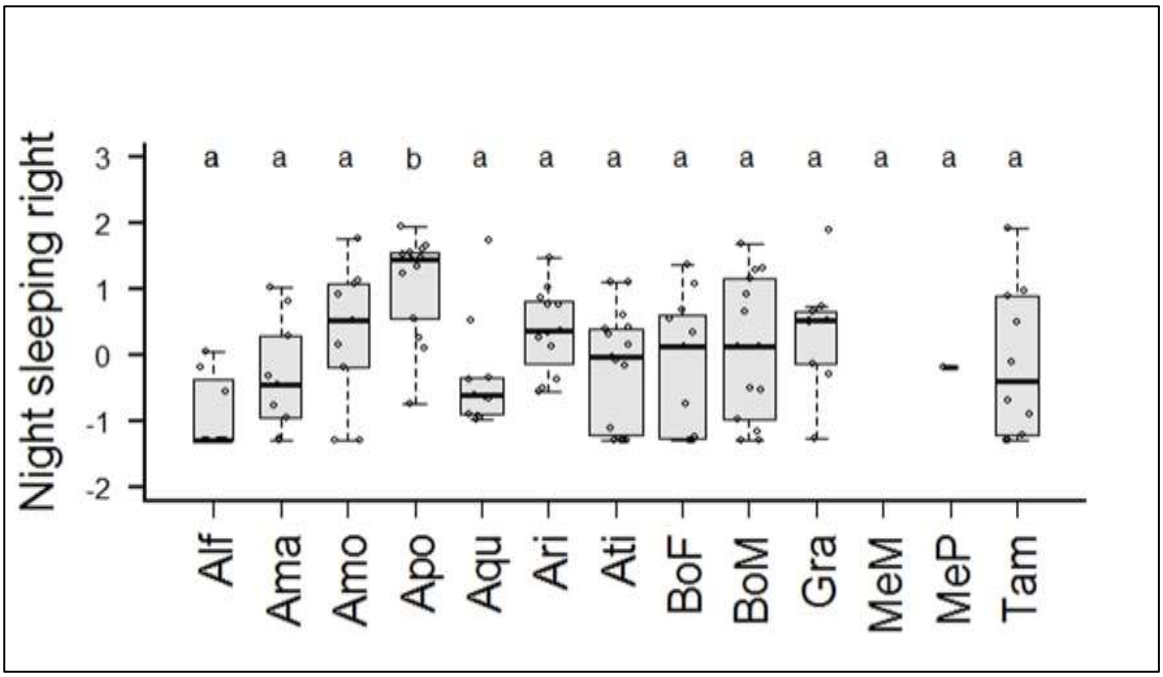

Figure 16. Individual variation of sleeping in the position “sleeping right” recorded during 160 nights for the observed dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. As for above lower case letter indicates significant variance at $p<0.05$.

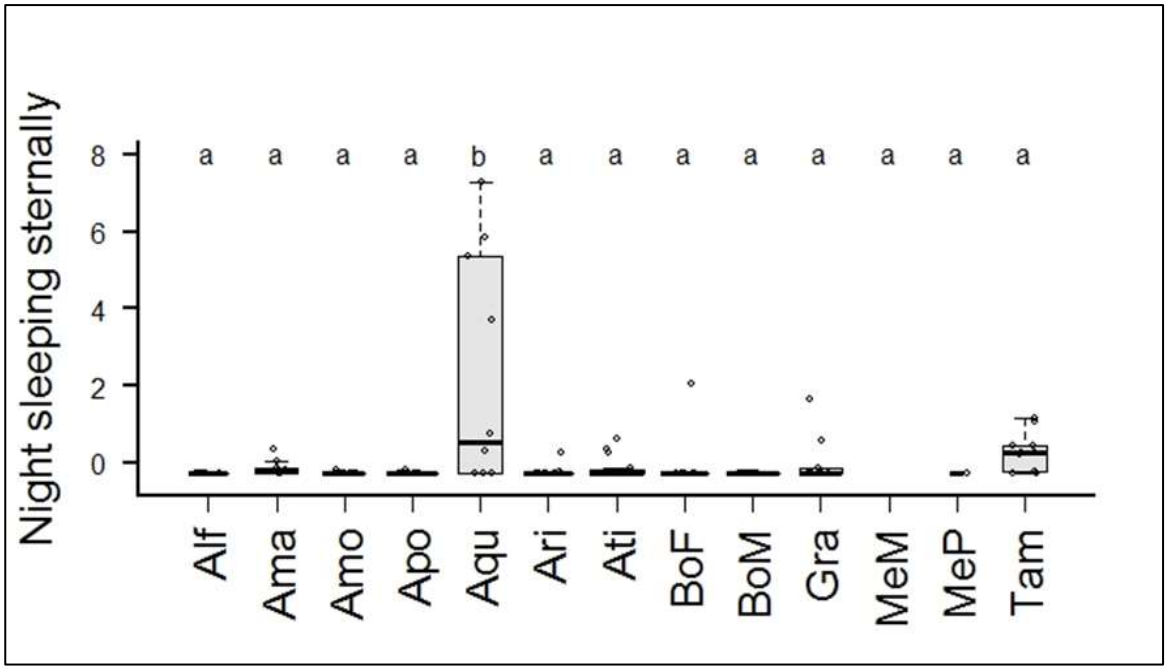

Figure 17. Individual variation of sleeping in the position “sleeping sternally” recorded during 160 nights for the observed dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. As for above lower case letter indicates significant variance at $p<0.05$.

2.5 Discussion

2.5.1 Overall sleep and activity profiles of dogs

On average, the dogs in our study slept 370 minutes (64.9% ± 25.9) of the time during the night. Compared to other studies, this amount time is significantly less than previous reported (e.g. **660 min** Owczarczak-Garstecka & Burman, **720 min** 2016; Zanghi et al., 2013b). Moreover, similarly to the study of Owczarczak-Garstecka & Burman (2016), the greatest difference observed was in the amount of time spent sleeping during the day. While other studies reported that dogs will spent somewhere between 20-40% of daytime asleep (Takeuchi & Harada, 2002; Zanghi, Kerr, DeRivera, Araujo, & Milgram, 2010; Zanghi et al., 2013), our dogs slept only 6% of the time, and not all the dogs exhibited this behaviour.

Nonetheless, the amount of time spent inactive during the day was higher than any other activity, which may indicate that the motivation for sleeping it is present, but is prevented, probably caused by external factors, such as the kennel environment (e.g. noise of humans nearby). Owczarczak-Garstecka & Burman (2016) reported the main reason why the dogs in their study were not sleeping

during the day was due to kennel practices. The kennel our dogs were maintained is located nearby a busy road and several University buildings, with students and staff passing often by the kennels. As there are no physical barriers other than a wired fence between the kennels and the street, the dogs were constantly exposed to multiple sources of disturbances, and these disturbances, may have prevented them from sleeping during the day.

Moreover, in the same area as the dogs, the building of animal experimentation is located, and since rodents are the most common species used for research in the university, students are often at the site, even after normal workings hours.

Studies have shown that constant barking and noise are both crucial factors that reduce dog welfare, especially in environments such as shelters and kennels (Hewison, Wright, Zulch, & Ellis, 2014a; Sales, Hubrecht, Peyvandi, Milligan, & Shield, 1997). Therefore, the dogs in our studies may be affected by the on-going activities nearby to the kennels and more studies should be done to assess the influence of the environment on their sleep.

Another interesting result found in our study was the number of sleeping bouts recorded. Although at first our numbers seemed high (range 5 to 23), it was actually less than reported in the literature (e.g. **23** in Adams & Johnson, 1993; **32** Owczarczak-Garstecka & Burman, 2016; **60** Takeuchi & Harada, 2002). This may be connected to an altered sleep architecture due to the irregular patterns of sleep; since they cannot sleep during the day, they sleep more continuously and in longer bouts during the night, instead of several bouts throughout the night and day, as it would be expected.

In humans and other animals, sleep deprivation causes a rebound effect, with the motivation for sleeping increasing when several episodes of reduced sleep are present (Orzeł-Gryglewska, 2010) . Moreover, it has been observed that prolonged wakefulness results in longer bouts of sleep and decreases the number of spontaneous awakenings during subsequent episodes of sleep (Guillaumin et al., 2018; Langford & Cockram, 2010).

The short latency between wakefulness and the event of the first sleep bout in our population may indicate motivation to sleep as soon as the major activities of the day ceased. As the first episode of sleep at night regulates physiologically the body for sleep and increases sleep motivation (Schwartz & Roth, 2008), we observed that the majority of bouts (fragmentation) occurred early in the night , with the other events of sleep being longer and consistent in the later hours of the night. Moreover, dogs were observed to waken up only to perform maintenance behaviours or to bark, similarly to previous studies (Adams & Johnson, 1994a).

Although not a consistent result, it was still possible to verify that some individuals had their sleeping patterns disrupted as a subsequent effect of losing sleep in the first night they were monitored. As sleep is a homeostatic process, the first effect of sleep loss is sleep rebound, as to restore homeostasis (Vyazovskiy & Tobler, 2012). For this to happen, the brain will initially increase the number of slow-wave (SWA) sleep bouts, and REM sleep may not be restored until several nights later (Banks & Dinges, 2007). However, if the restored sleep has any episodes of awakenings, even short ones, another rebound effect happens, enhancing SWA, which can then progress as an accumulative deficit of REM, thus causing subsequent episodes of sleep deprivation (Brunner, Dijk, Borbeiy, & Borbély, 1993; Vyazovskiy, 2015).

Kis et al (2014) found in their study that sleep deprived dogs, even for short periods during the day, would have short latency to the first sleep bout at night, would spend more time sleeping and have longer bouts of SWA sleep, which corroborates with the patterns we found in our dogs.

Differences in sleeping patterns were also found in the number of the bouts between males and females and dogs of different age in the present study, with females and older dogs sleeping longer than males and younger dogs.

A recent study showed that male and female dogs have differences in their sleeping patterns, with females having more sleep spindles[1] during NREM sleep than males, which increased with age (Iotchev et al., 2019). This is also true for

[1] Oscillations of neural activity produced in the thalamic nuclei region of the brain and specific to the NREM phase of sleep. They are called spindles due to the patterns they form in the EEG. See de Genaro, & Ferrara (2003).

humans. Women have almost double number of spindles than men, sleep significantly longer and have better sleep efficiency, nonetheless they are also 40% more susceptible to insomnia than the opposite sex (Mallampalli & Carter, 2014; Mong & Cusmano, 2016).

In rodents, it is also possible to observe differences between males and females, although, in this case, males are the longer sleepers. Yet, after episodes of sleep deprivation females have the ability to recover more efficiently than males by displaying longer bouts of slow-wave sleep (Andersen et al., 2008; Koehl, Battle, & Meerlo, 2006).

The female dogs in our study did sleep significant more than males (had less bouts) and Tammy and Grace were recorded sleeping for longer during the day. Correspondingly to humans and rodents, it appears they had better rebounds of sleep and were able to maintain longer bouts, which in itself is a form of improving sleep quality (Schwartz & Roth, 2008). Thus, it is possible that male dogs are more affected by the effects of sleep deprivation, as biologically they were unable to restore sleep as efficiently as females. Nevertheless, further studies are needed to test this hypothesis.

The effects of age were also a factor contributing to differences in sleeping patterns, with older dogs in our study sleeping longer and having more bouts than younger dogs, but only during the day. Ageing is one of the main biological factors affecting sleep. Both animal and human studies have shown ageing is associated with changes in sleep quality and quantity, increased sleep fragmentation, increased time spent in NREM sleep during the day and decreased bouts of REM sleep at night (McKillop et al., 2018; Nakamura, Takasu, & Nakamura, 2016).

Research has shown that there is a difference in sleeping patterns according to age in dogs; this difference is most perceived during the day. Older dogs sleep longer, have more bouts and are more inactive during the day than younger dogs (Takeuchi & Harada, 2002; Zanghi, Kerr, de Rivera, Araujo, & Milgram, 2012b; Zanghi et al., 2013). Furthermore, older dogs exhibit less REM sleep during the night, longer NREM bouts during the day and increased wakefulness during the night, which corroborates with studies in humans, rodents, and our results.

However, night bouts tend to decrease with age, with extended sleep duration (Zanghi, 2010), hence the reason why we could not find any significant differences between older and younger dogs' sleep at night in our study.

2.5.2 Sleep positions

It was possible to identify significant variations in the preferences on the positions adopted for sleep both at the population and at the individual level.

Sleeping in lateral recumbency was the most common position adopted by the dogs in this study. This position it is believed to be the most common posture adopted by mammals in general (Hediger, 1980). In humans, sleep laterality has been found to have association with several physiological measures, sleep disorders, stage of development and even personality. Furthermore, sleep deprivation or those suffering from any difficulties to sleep, such as insomniacs have more fragmented sleep episodes and consequently shift positions more during sleep than normal sleepers do (Agargun, Boysan, & Hanoglu, 2004; Lorrain & De Koninck, 1998).

Investigations with dogs have mixed results regarding laterality, although studies have found lateralization in locomotor patterns, preferences in paw used to play, retrieving a command and even a bias response of nostril preference (right side) while investigating a novel stimulus (Ocklenburg, Isparta, Peterburs, & Papadatou-Pastou, 2019; Siniscalchi, d'Ingeo, & Quaranta, 2017; Tomkins, Williams, Thomson, & McGreevy, 2012). Moreover, studies also find connections between dogs' personalities and laterality (Schneider, Delfabbro, & Burns, 2013) and that ambilaterality can be a side effect of stressful environment, with most dogs living in normal conditions having paw preferences (Barnard, Wells, & Hepper, 2018; Salgirli Demirbas et al., 2019).

In a study focusing on the effect of lighting conditions on dogs' sleep, it was possible to verify that depending on lighting, dogs will sleep in different postures. Sternal recumbency was most common observed under "lights on" conditions (most

during the day) versus lateral recumbency when there was no light (nocturnal sleep cycles) (Fukuzawa & Nakazato, 2015).

Recently a study investigated the possibility of laterality in sleep postures in shelter dogs. While the results showed no preference for sleeping on a specific side, it was clear that dogs would wake up several times during the night to change their sleeping position. The author suggested that the environment of the shelter, as observed in other studies, could be an interference with sleep (Houpt, 2019).

In the above-mentioned studies, dogs spent most of their time in lateral recumbency, which corroborate with our results. In addition, the sleeping postures of the dogs were affected by the number of times the dogs woke up during the night, which also was found in our studies. Although further investigation is necessary, it seems that lateral recumbency is not only the preferable sleeping position for dogs, but also is the position that allows the individuals to achieve better sleep efficiency.

Sleeping on their back seems to be related to the preference of some young individuals. These may be justifiable by two conditions: first, sleeping on the back has a different physical demand than sleeping in a more relaxed posture, which could cause discomfort in older dogs. Second, as it is a posture that fully exposes the belly (and vital organs); most individuals could be uncomfortable to sleep in such a risky posture.

2.6 Conclusion

Dogs in our study dogs slept substantially less than previously reported. Moreover, the sleep architecture is different from the patterns observed in other studies, with several awakenings during the night, but with almost no sleep recorded during the day. Sleep was also different between sexes and age groups, and the fragmentation of sleep interfered on the preferred sleeping position.

The altered sleep patterns appear to be a direct reflect of the dogs' environment. The increase of rest during the day can be an attempt to restore energy balance, since diurnal sleep was unachievable due to kennel practices. It

also seems that the dogs adapted their sleeping routines by sleeping earlier, waking up earlier, and having longer sleeping bouts at night.

Since sleep is an important component of homeostatic control, it is important to understand the way it influences dogs' behaviour and welfare, and these matters will be further explored in the following chapters.

It is important to reinstate that due to the statistical limitation found in our models when including kennels as a variable, pseudoreplication might be occurring in relation to dogs' behaviour and affecting results' outcomes. Hence, further investigations should be carried for a better understanding of the impact of sleep disruption in the individual dogs.

Chapter 3. How sleep fragmentation affects overall behaviour in kennelled laboratory dogs

3.1 Introduction

Activity and rest patterns have been thoroughly studied in humans and other animal species to characterize the biology of sleep (Adan et al., 2012; Rial et al., 2010). Aside from the endogenous responses (e.g. hormones), biological rhythms in mammals are also a consequence of the animal alternating between sleep and wakefulness to perform necessary behaviours (such as feeding and mating). In this way, bodily functions and behavioural patterns are synchronized with the day and night cycles (Mader & Mader, 2016; Randler, 2014). Consequently, sleep influences behaviour and in turn behaviour can influence sleep (Banks & Dinges, 2007; Tougeron & Abram, 2017).

Domestic dogs possess a clear diurnal pattern of activity and have sleeping cycles comparable to humans (Nishino, Tafti, Sampathkumaran, Dement, & Mignot, 1997; Piccione et al., 2014; Zanghi, Kerr, DeRivera, Araujo, & Milgram, 2010), whereas feral canids express more sleep during the day and an increase in activity during the dark phase (Boitani & Ciucci, 1995; Miklósi, 2016). Dogs will spend over 60% of the light phase awake. Similarly, they will concentrate their total locomotor activity (60-75%) to the same phase and the levels of activity will fluctuate according to environmental variables such as feeding regime and light onset (Fukuzawa & Nakazato, 2015; Nishino et al., 1997; Piccione et al., 2014; Takeuchi & Harada, 2002; Brian M. Zanghi et al., 2012b).

Sleep deprivation has been proven to impair circadian rhythms (Touitou, Reinberg, & Touitou, 2017a). In humans, sleep deprivation compromises motor and cognitive diurnal functions, increases anxiety and depressive related behaviours, affects overall mood, consumption behaviours and alters emotional responses (Hudson et al., 2019; Orzeł-Gryglewska, 2010; Gabriel Natan Pires, Bezerra, Tufik, & Andersen, 2016). In non-human animals, sleep deprivation has been observed to affect locomotion patterns and general activity (**mouse:** Guillaumin et al.,2018; **cow:** Hänninen, 2007; **rat:** Hsieh et al., 2014; Storch, Höhne, Holsboer, & Ohl, 2004; **dog:** Tobler & Sigg, 1986; **chicken:** Yngvesson et al., 2017); to increase the

display of anxiety like behaviours (e.g. excessive self-grooming, **rat:** Pires, Tufik, & Andersen, 2013), to increase aggressiveness (**rat:** Hicks, Moore, Hayes, Phillips, & Hawkins, 1979), to decrease cognitive performance (**review on mammal, birds and some invertebrates:** Vorster & Born, 2015), alter mating patterns (**tits:** Greives et al., 2015; **dormouse:** Kukalová, Gazárková, & Adamík, 2013), migratory patterns (**thrush:** Fuchs, Haney, Jechura, Moore, & Bingman, 2006) and even affect communication between conspecifics (**bees:** Klein, Klein, Wray, Mueller, & Seeley, 2010).

In dogs there have been investigations of their activity/rest cycles associated with feeding regimes, activity, age, sex, the environment and even with their affective states (Adams & Johnson, 1994; Bunford et al., 2018; Iotchev et al., 2019; Kis et al., 2017; Zanghi, Kerr, de Rivera, Araujo, & Milgram, 2012b). However, little is known about how sleep itself and, especially how sleep deprivation affects the general behaviour of the species. Therefore, this chapter focused on investigating the effects that non-induced sleep fragmentation and sleep loss have on the diurnal behaviours of laboratory dogs.

3.2 Aim

To investigate whether dog sleeping patterns and sleep fragmentation affected the overall behaviour of dogs.

3.3 Methods

3.3.1 Subjects and study site

The experiment was carried with the same dogs and setting as described in Chapter 3, sections 3.3.1. and 3.3.2.

3.3.3 Data collection of behavioural data

Dogs' behaviour were assessed during 24-hour observations in five-days sessions between October 2017 and May 2018. The methods used to record the behaviours were carried out as described in Chapter 3, section 3.3.3.

3.3.4 Statistical analysis

All behavioural data were explored graphically and tested for normality using Anderson-Darling tests. All statistical tests were considered significant at $p<0.05$. Descriptive statistics of all the analysed metrics were conducted and results are presented as either counts or percentages with a standard deviation.

To understand how sleep affected the dogs' behaviour, multiple linear model tests were constructed (GLMMs, lmer function for data with normal distribution and glmer for non-normal data, both with lme4 package in R; Crawley, 2007). The selection of the reported results of the GLMM was made using the minimum model criteria. Significant levels between expressed behaviours were then found using pair-wise comparison (Crawley, 2007).

Duration of sleep during the night and the number of sleep bouts during the night were collinear. Therefore, the number of sleep bouts during the night was selected as the primary measure of sleep, since sleep loss at night can have a direct effect on activities during on the following day. Sleep duration and the number of

sleeping bouts recorded during daytime observations were not collinear; hence, both variables were included in the model.

Due to the metrics difference between variables, data were scaled (Becker et al., 1988). Further details of how the models were constructed can be found in Chapter 3, section 3.3.4.

3.4 Results

3.4.1 Characteristics of behavioural activity of the dogs

A total of 325 hours of diurnal behaviour was analysed and descriptive statistics for measures of activity and rest were calculated for each dog. Overall, dogs spent most of the day exhibiting inactivity-related behaviours (61.31%), followed by locomotion (12.23%), sleeping (6.03%) and exploring (5.22%). All other behaviours were recorded less than 5% of the observations (Figure 18).

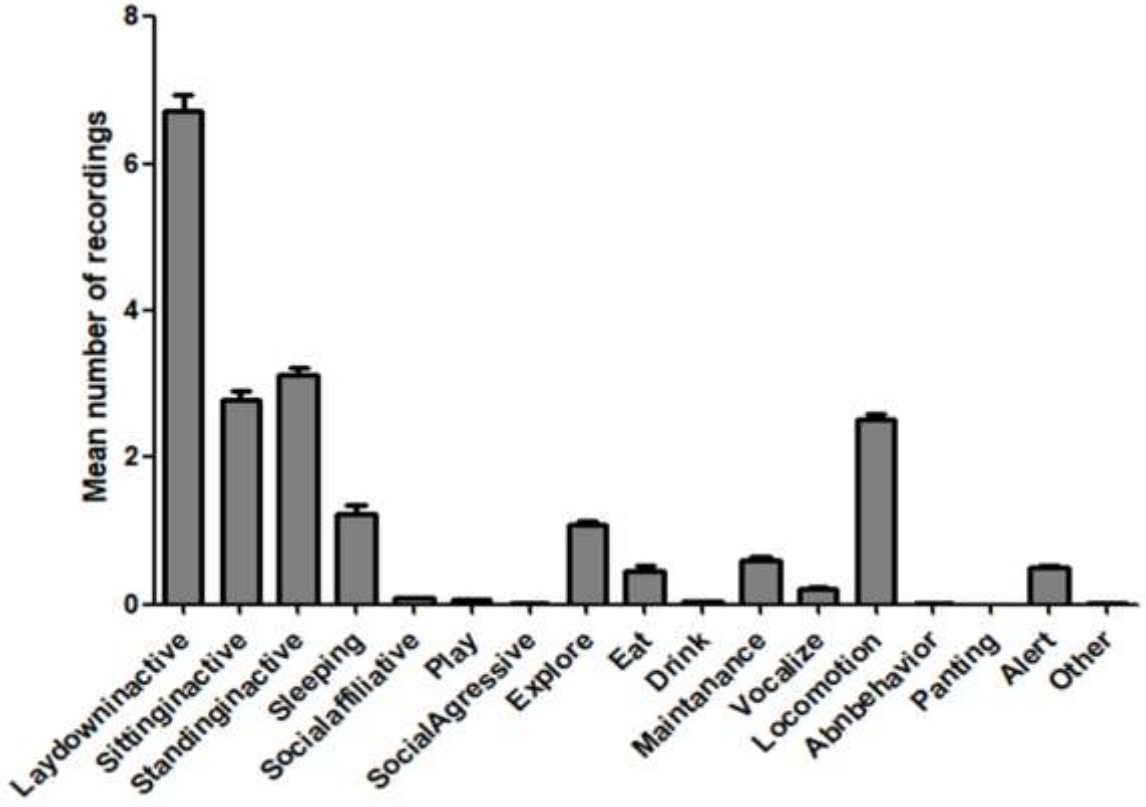

Figure 18. Mean expression of different behavioural categories during diurnal observations of laboratory dogs kennelled at the Federal University of Ouro Preto, Brazil. Error bars show ± standard deviation of the mean.

3.4.2. Influence of sleep metrics on behaviour

In general, the fragmentation of sleep during the night was responsible for increased inactivity during the night ($t = 3.213$, $p < 0.001$), and increased time spent laying ($z = 20.412$, $p < 0.001$) and time spent standing inactive ($z = 8.812$, $p < 0.001$) during the day. It also increased time spent eating ($z=2.488$, $p<0.01$), in locomotion ($z = 11.037$, $p < 0.001$), and increased the display of maintenance behaviours ($z = 3.400$, $p < 0.001$).

Furthermore, the increase in sleep fragmentation diminished the expression of play behaviour ($z = -2.042$, $p < 0.05$), and the amount of time the dogs were alert ($z = -5.333$, $p < 0.001$). The fit models' results are summarized in Table 7.

As expected, the amount of time the dogs spent asleep during the day decreased the expression of most behaviours, except for the time spent eating which increased when dogs slept for longer ($z = 2.020$, $p < 0.05$). In contrast, the number of diurnal sleeping bouts had a mixed effect on behaviours. It increased the number of times the dogs were recorded sleeping ($z = 36.51$, $p < 0,001$), or alert ($z = 4,585$, $p < 0,001$), decreased the amount of eating ($z = -5,797$, $p < 0,001$) and was the only variable, which had an effect on the vocalization, which decreased when the bouts were higher during the day ($z = -3.826$, $p < 0.001$).

Table 7. GLMM results for the optimal models describing the relationship between laboratory dogs' behaviours and sleep metrics (independent variables)

Behaviour	Independent variable	Estimate ± SE	Coefficient value	p	Error distribution
Alert	Sleep bouts (night)	-0.0529 ± 0.0099	-5.333	$9.68e^{-08}$	Poisson
	Sleep bouts (day)	2.6647 ± 0.5811	4.585	$4.54e^{-06}$	
	Sleep duration (day)	-8.2486 ± 1.9818	-4.162	$3.15e^{-05}$	
Eating	Sleep bouts (night)	0.0243 ± 0.0097	2.488	0.012	Poisson
	Sleep bouts (day)	-1.5813 ± 0.2728	-5.797	$6.75e^{-09}$	
	Sleep duration (day)	0.1431 ± 0.0099	2.020	0.043	
Inactive	Sleep bouts (night)	0.0597 ± 0.0185	3.213	$1.31e^{-03}$	Gaussian
Lay Down	Sleep bouts (night)	0.0383 ± 0.0025	15.294	$< 2e^{-16}$	Poisson
	Sleep bouts (day)	0.4927 ± 0.0262	18.786	$< 2e^{-16}$	
	Sleep duration (day)	-0.0002 ± 0.0000	-6.657	$2.8e^{-11}$	
Locomotion	Sleep bouts (night)	0.0426 ± 0.0039	11.037	$< 2e^{-16}$	Poisson
	Sleep bouts (day)	-0.7863 ± 0.1042	-7.550	$4.36e^{-14}$	
	Sleep duration (day)	-0.0974 ± 0.0453	-2.151	0.031	
Maintenance	Sleep bouts (night)	0.0266 ± 0.0078	3.400	$6.75e^{-4}$	Poisson
	Sleep bouts (day)	0.1326 ± 0.0900	1.473	ns	
	Sleep duration (day)	0.0327 ± 0.0327	0.858	ns	
Play	Sleep bouts (night)	-0.0397 ± 0.0194	-2.042	0.041	Poisson
	Sleep duration (day)	-3.6741 ± 2.4396	-1.506	0.132	
Sleeping (day)	Sleep bouts (night)	-0.0115 ± 0.0068	-1.706	ns	Poisson
	Sleep bouts (day)	1.1478 ± 0.0314	36.521	$< 2e^{-16}$	
	Sleep duration (day)	0.2570 ± 0.0126	20.348	$< 2e^{-16}$	
Sitting	Sleep bouts (night)	0.0028 ± 0.0104	0.269	ns	Poisson
	Sleep bouts (day)	-0.3648 ± 0.1888	-1.933	0.053	
	Sleep duration (day)	-0.2914 ± 0.0990	-2.943	0.003	
Standing	Sleep bouts (night)	0.0303 ± 0.0034	8.821	$< 2e^{-16}$	Poisson
	Sleep bouts (day)	-0.5434 ± 0.0952	-5.707	$1.15e^{-08}$	
	Sleep duration (day)	-0.2084 ± 0.0614	-3.393	0.001	
Vocalizing	Sleep bouts (night)	0.0039 ± 0.0137	0.287	ns	Poisson
	Sleep bouts (day)	-0.7979 ± 0.3449	-2.313	0.027	
	Sleep duration (day)	0.0847 ± 0.1452	0.584	ns	

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

3.5 Discussion

3.5.1 Influence of sleep on the behaviour of the dogs

Inactivity was the behaviour most observed while collecting data from the diurnal observations of the dogs. As previously discussed in Chapter 3, the shifted sleep architecture of the dogs in our study, which almost never sleep during the day and have less fragmented sleep than previously reported, might be leading to an increase in inactivity during the day. The same response was found by Owczarczak-Garstecka & Burman (2016) for shelter dogs. According to their research, their dogs also presented a small percentage of sleep during the day and were observed being inactive for most of the time.

Dogs are predominantly diurnal when housed in homes and kennels, which means they will express more activity during the day and concentrate a higher proportion of sleep (especially REM-sleep) during the night (Piccione et al., 2014; Zanghi, 2010). Hence, it is not expected to observe elevated inactivity as part of their normal behaviour. The increased time spent inactive both during the day and at night is related to the effect that sleep deprivation has over the patterns of normal circadian distribution of activities (Clodoré, Benoit, & Foret, 1986; James, Honn, Gaddameedhi, & Van Dongen, 2017b).

Sleep loss caused by sleep fragmentation increases daytime somnolence, tiredness and fatigue, which limits physical activity (Kredlow, Capozzoli, Hearon, Calkins, & Otto, 2015). Sleep loss also causes energy imbalance, affects hormone production, changes metabolism function and triggers stress responses that may lead to reduced activity as the body tries to restore homeostasis (Knutson, Spiegel, Penev, & Van Cauter, 2007c; Orzeł-Gryglewska, 2010).

Recently, the association of sleep and activity levels was investigated in dogs and the sleep macrostructure of individuals changed between active (6-8h of activities/social interactions) and passive days. Pre-sleep activity increased time spent sleeping, and time in REM-sleep, reduced the latency to first sleeping bout and promoted less awakenings during subsequent sleep, which increases sleep efficiency (Bunford et al., 2018; Kis et al., 2014b).

As the dogs in our study displayed an altered sleep structure, we can identify a cyclic effect – the disruptive pattern of sleep at night, makes them less motivated or able to remain active during the day, and, as consequence, the lack of activity during the day does not promote a better sleep at night. This could also be the reason why our results displayed increased locomotion as an effect of increased sleep fragmentation.

Although the study from Bunford et al (2018) showed a positive change in sleep structure after an active day for dogs, this assessment was done over a short period of time. In humans, it has been found that regular physical activity, opposed to extreme activity, is the most beneficial to sleep (Kredlow et al., 2015). Thus, as inactivity is still the main behaviour observed during the day in our study, probably, the benefit of more movement was not enough to improve sleep. Correlated to this scenario, two other behaviours – play and alert– were also affected by the increased episodes of sleep fragmentation.

Sleep deprivation has been associated with reduced cognitive performance on daily activities in humans and several animal species; even after only one night of sleep deprivation (Hudson et al., 2019; Vorster & Born, 2015). Dogs had their ability to perform tasks tested recently after episodes of sleep deprivation, and the result was similar to that found in the literature, they perform poorly, as they lack attention (Iotchev et al., 2017). Being alert is a state that goes beyond mere vigilance, since it demands more of our cognitive capacities to generate responses to the signals sent by the environment. As verified in previous studies, sleep deprivation would interfere in the ability to respond; therefore, it is feasible that our dogs are less responsive after several episodes of sleep loss.

Nonetheless, as previously discussed, the environment in which the dogs are housed has various stimuli that may be interfering with rest during the day (e.g. noise and staff activity). In this case, perhaps lack of attention to environmental events is a way of mitigating these adverse conditions to try to rest, even if unsuccessful. Further discussion on the condition of the kennels can be found on Chapter 4.

Play was also diminished after increased sleep fragmentation. Dogs, differently to other mammal species, continue to exhibit play behaviour as adults and evidence suggest its expression cannot be considered a mere by-product of biological process, as the behaviour is consistent over time. Moreover, in adult dogs research indicates that play is a behavioural state that serves multiple functions such as: enhancing motor skills, improving social cohesion and even as coping mechanism to deal with challenges faced in the environment (Bradshaw, Pullen, & Rooney, 2015; Sommerville, O'Connor, Asher, O'Connor, & Asher, 2017). Play has also been used as a new measure of animal welfare, since its presence, and especially, its absence, is intrinsically connected to the individuals emotional state and it adaptation to the environment (Ahloy-Dallaire, Espinosa, & Mason, 2018; Held & Špinka, 2011). So far, play has not been investigated in a direct relation to sleep, but as it is a form of exercise, we could expect that sleep deprived individuals would not expend energy to play.

Despite this, a recent study with dogs showed that when the individuals tested experienced a negative emotion during the day (represented in the study by a negative social experience with a strange human), their architecture of sleep changed compared to days with positive social interactions (Kis et al., 2017). This could mean that behaviours that allow animals to experience release of endorphins, such as positive play, also contribute to a better sleep.

The dogs in our study did not display play behaviour often while inside the kennels. Aside from this situation, dogs had play sessions once a week, where all the dogs were allowed to play together (although it was not possible to quantify play during these sessions, as it was off-camera). Nonetheless, no change in sleeping patterns was observed between days of the week (no difference from one night to the other as reported in the previous chapter), which indicates that play sessions were not contributing to sleep quality. Moreover, our results found an inverted relationship between play and sleep, which strengthens the assumption of sleep having an impact on the motivation to play.

Lastly, one of the most prominent results in our study was the direct association of feeding behaviour with sleep

Feeding was the only behaviour that was equally associated with sleep loss at night and during the day. Sleep latency, sleep fragmentation and sleep dysfunctions (such as increased sleepiness) during the day are considered catalysts to increase food consumption and energy intake during the day, being directly responsible for changes in the metabolism leading to increased body weight and adiposity (Knutson et al., 2007c; Penev, 2007) .

Changes in the macrostructure of sleep and disrupted circadian rhythms are associated with weight oscillations of individuals due to characteristics of metabolic modulators that maintain energy imbalance, as those are similar to the ones that control vigilance and sleep. Hence, altered sleep patterns may affect the body´s energy distribution processes causing either over-expenditure or accumulation of energy (Horne, 2009; Mavanji, Billington, M. Kotz, & Teske, 2012).

In dogs, the association between feeding times and sleep has been investigated before. Two studies compared dogs from different ages to different feeding regimes and were able to determine that frequency of feeding and feeding time had significant impact in sleep structures (Zanghi et al., 2012, 2013). Shifting the feeding regime for the studied dogs from a single feed to being fed twice a day resulted in early onset of sleeping bouts at night and of activity in the following morning. It also resulted in shorter latency to sleep in the dark phase. Furthermore, dogs changed the quantity of sleeping bouts during the day, with less bouts, but for longer times, maintaining the same sleep levels that were found when fed once a day (Zanghi et al., 2012, 2013).

Shifting feeding times has deleterious effect to the organism due to metabolic confusion, which occurs because of desynchronization of internal clocks. Sleep loss reduces activity and increases sleepiness during the day, which change the patterns of appetite (Knutson et al., 2007c; Mavanji et al., 2012). It was also observed in rats that actively consuming food in rest phases changes the rhythms of glucose homeostasis reflecting in further changes in muscular activity (Opperhuizen et al., 2016). As glucose is one of the main components regulating energy imbalance, it is understandable why such changes would have deleterious effects on the metabolism.

For our dogs, food was given once a day in the morning, but in an amount greater than the dogs usually consumed, and the remaining food was available throughout the day and additional food was added during cleaning times, if necessary (i.e. they were *ad libitum* fed). Thus, it was possible to observe a peak of feeding in the morning, followed by smaller events of feeding, without a specific pattern, throughout the day. Since most of the dogs also did not sleep during the day, it is possible that their rhythms have been disturbed by sleep suppression, but further desynchronization was potentially caused by the absence of specific feeding times and the amount of food consumed (individuals might be overfeeding or underfeeding). Zanghi (2013) suggested that feeding patterns do influence the locomotor behaviour of dogs and consequently their sleep. Managing the feeding properly might contribute for a better quality of sleep in the dogs in our experiment.

3.6 Conclusion

Quantity and quality of sleep directly affected the dogs' behaviour, which became more inactive, ate more, played less and were less alert during most part of the diurnal observations. The most recorded behaviour in our dogs was inactivity, and our results indicate this increase in inactivity is directly associated with their sleeping patterns at night.

Sleep fragmentation appears to leave the animals tired and more irresponsive to the environment. Even with locomotion increasing during the day after more fragmented nights, the amount of exercise was not sufficient to increase sleep duration, and consequently, provide a better sleep quality.

The observed eating habits of the dogs may also signal that main metabolic functions were being compromised by the altered sleeping patterns, which could cause further disruptions to the sleeping cycles. Changes in behavioural patterns are associated with individuals' quality of life and can be used to measure adaptability to the environment. Thus, changes in behaviour related to lack of sleep, especially the high patterns of inactivity may indicate a compromised welfare for the dogs in our study. Further investigations using physiological and environmental

measures could help address the question of whether sleep is an important tool when verifying the welfare of laboratory dogs in relation to their environment.

Chapter 4. Do environmental conditions affect sleep in kenneled dogs?

4.1 Introduction

Evolutionarily, animal species are selected in relation to the environment in which they live and, consequently, evolved specific behaviours to match these conditions, often resulting in active behaviours being expressed at a specific time of day (Morgan, 2004). These fluctuations in activity patterns are connected to the mechanism known as circadian rhythmicity, a process modulated by external triggers (e.g., light levels) that generate endogenous responses from the organism, allowing better adaptation to the environment (Adan et al., 2012; Morgan, 2004; Randler, 2014). Circadian rhythms are an intrinsic part of the homeostasis process; any disruption of an animal's biological clock, such as caused by sleep deprivation or stress, directly compromise the health of individuals (Tahara et al., 2015; Takahashi, Hong, Ko, & McDearmon, 2008; Touitou, Reinberg, & Touitou, 2017b)

Comparable to the circadian clock, sleep has an important part to play in helping animals to adapt to their environment and this is evident by the cost that sleep has: being inactive for so long would not be adaptive, hence sleep as any other behaviour, has an important function (Mader & Mader, 2016; Randler, 2014; Roth, Rattenborg, & Pravosudov, 2010). Despite this, sleep has been investigated in most animal species under laboratory conditions, without the full consideration of its ecological value and without considering the importance that different ecological variables may have on sleep quality (Aulsebrook et al., 2016; Tougeron & Abram, 2017).

The main external trigger that modulates sleep is light; the presence or absence of not only luminous emittance, but of light of different wave lengths, such as blue light, is the cue to the suprachiasmatic nucleus region of the brain that is time to go to sleep (Dijk & Archer, 2009; Dominoni, Borniger, & Nelson, 2016). However, daylight is not the only ecological trigger that affects sleep; other components such as artificial lighting (Dominoni et al., 2016), sound (Muzet, 2007), temperature (Harding, Franks, & Wisden, 2019), activity during biological rest phase (Nunez, Yan, & Smale, 2018) and even the presence of ectoparasites (Christe,

Richner, & Oppliger, 1996) influence the duration and fragmentation of sleep. Environmental studies have shown that nocturnal artificial lights have negative impacts on an array of wild species, changing the timing of their behaviour, affecting mating season and decreasing reproductive success (Dominoni et al., 2016; Dominoni, Carmona-Wagner, Hofmann, Kranstauber, & Partecke, 2014; Robert, Lesku, Partecke, & Chambers, 2015). In captive/laboratory settings further research demonstrates that artificial light also disrupts the circadian cycle (Ishida et al., 2005), affects sleep quality and quantity (Abou-Ismail et al., 2008; Raap, Pinxten, & Eens, 2016), results in changes in body mass and feeding habits (Ferter & Meyer-Rochow, 2010; Raap, Casasole, et al., 2016). It also induces chronic stress, which can result in brain damage and depressive states (Abou-Ismail et al., 2008; Tapia-Osorio et al., 2013).

Sound pollution is considered the second largest environmental cause of health problems in humans (World Health Organization, 2011). Furthermore, noise exposure during the night causes the body to take longer to fall sleep, causes sleep fragmentation and increases stress responses (Hume, Brink, & Basner, 2012; Muzet, 2007). In the long term, cortisol production remains elevated due to noise, which can lead to cardiovascular diseases (Münzel, Gori, Babisch, & Basner, 2014). Likewise, studies in animals have shown several deleterious effects of noise on animals' health, and comparable to humans, a number of species have increasing cortisol production due to noise exposure (as reviewed by Kight & Swaddle, 2011). In zoos, research has found that the presence of the public can alter the soundscape of the environment and increase the levels of decibels around the enclosures (Quadros, Goulart, Passos, Vecci, & Young, 2014). Noise disturbance leads species to change their behaviour: makes them more anxious, increases aggressiveness, decreases social contact and reflects directly in elevated stress levels (Davey, 2007; Jakob-Hoff et al., 2019; Quadros et al., 2014).

Temperature is another component that can shift sleeping patterns. Body temperature can change because of external temperature (heat and cold), but also due to circumstances such as pain, stress, state of nutrition and imminent risks, which will trigger different responses in the brain that may interfere with the sleep/wake cycle (Harding et al., 2019; Romeijn et al., 2012). Non-REM sleep

seems to be triggered by an association of body cooling and seeking the comfort of a warm place to sleep (e.g. beds in humans, nests in primates and rats), while sleeping in very low temperatures will cause sleep debt (e.g. hibernating mammals) (Harding et al., 2019). However, while warmth may induce sleep, only the heating of skin temperature is beneficial. Research has shown that insomniac humans have elevated core temperature, signalling that elevated metabolic rates producing heat in association with warmer environments could cause sleep disruption (Caddick, Gregory, Arsintescu, & Flynn-Evans, 2018; Harding et al., 2019). This is also observed in birds, where the elevation of temperature at night induces sleep fragmentation (Caorsi, Sprau, Zollinger, & Brumm, 2019; Stuber, Dingemanse, & Mueller, 2017). Similarly, some mammals living in extreme thermal conditions, such as deserts, shift their sleeping patterns according to the change in temperature between seasons, sleeping less during summer *(Oryx leucoryx*; Davimes et al., 2018). Moreover, Javan slow lorises (*Nycticebus javanicus*) adjust sleeping patterns based on fluctuations of ambient temperature, and sleep duration is inversely proportional to the rise in temperature (Reinhardt, Vyazovskiy, Hernandez-Aguilar, Imron, & Nekaris, 2019).

All these environmental variables can interfere in sleep, either by directly affecting it, or by producing stress responses that can then interfere in sleep quality. Stress itself is known to be the main cause of sleep disruption and insomnia in humans (Jun & Polotsky, 2016; Van Reeth et al., 2000). The secretion of cortisol is also modulated by circadian rhythms, and it has an alternating cycle with melatonin, the sleep hormone (Buckley & Schatzberg, 2005). Sleep will be initiated when the activity of the HPA axis is at its lowest and sleep deprivation its associated with activation of the HPA axis; further hyperactivity of the HPA axis can lead to sleep problems (Buckley & Schatzberg, 2005).

In summary, stressful events will lead to the production of cortisol, which will inhibit sleep, causing delays in sleep on-set and increasing waking episodes during sleep cycles. In turn, sleep problems including insomnia and obstructive sleep apnoea can further increase HPA axis dysfunction (Buckley & Schatzberg, 2005; Jun & Polotsky, 2016; Sadeh et al., 2004; Van Reeth et al., 2000). The same response is found in most laboratory rodents; with stress being associated with poor

sleep quality and the combination of stress and sleep disruption leading to compromised immune function (Abou-Ismail et al., 2008; Pawlyk, Morrison, Ross, & Brennan, 2008; also review Revel, Gottowik, Gatti, Wettstein, & Moreau, 2009). Stress is also a main source of behavioural and physiological disturbances in most captive species (Brando & Buchanan-Smith, 2018; Wolfensohn et al., 2018). Sleep disruption can also be considered a stressor by itself, since lack of sleep compromises the whole system by overloading the adrenal function, suppressing immunity and can be responsible for depressive states (inability to cope with stressors) (Orzeł-Gryglewska, 2010; Tobaldini et al., 2016; Vandekerckhove & Cluydts, 2010).

Dogs in shelters are susceptible to multiple stressors sources, such as noise, visitors, co-specifics, veterinary procedures and normally have a history of coping with situations that contribute to poor welfare. In these individuals, the production of high levels of stress hormones, such as cortisol, reportedly triggers abnormal behaviours, aggression, diminishes the immune responses, and induces apathy (Protopopova, 2016). On the contrary, small changes in the management practices on shelters, such as restricted access to the dogs by visitors, lower noise levels, higher activity levels, and environmental enrichment (common practices to increase welfare) successfully decreased stress responses and even increased sleeping time in these animals (Hewison, Wright, Zulch, & Ellis, 2014b; Wells & Hepper, 2000; Brian M. Zanghi et al., 2013d)

The focus of this study was to identify and quantify different components of the environment that could interfere with dogs' sleeping cycles. We choose to do this study in laboratory kennel's environment because it is less variable (i.e., more environmentally stable) than in pet dogs' homes.

4.2 Aim

To investigate if different environmental factors in dog kennels affect sleep quality and sleep duration in dogs.

4.3 Methods

4.3.1 Study location and subjects

The data analysed in this chapter were acquired simultaneously with the data used to verify the sleep patterns of dogs from the Centre of Animal Science in Universidade Federal de Ouro Preto, Brazil. Thus, the subjects and the place of study remained the same as in the previous chapters. Detailed description of subjects and location are found in Chapter 3, sections 3.3.1. and 3.3.2.

4.3.2 Behavioural data

Dogs' behaviour was monitored during five-day assessment periods over the course of 8 months (from October 2017 to May 2018), totalling 130 days and nights. The methods used to record the behaviours are described in Chapter 3, section 3.3.3.

4.3.3 Biological material for hormonal measurement

The assessment of stress hormones in the dogs was conducted by a non-invasive measurement of glucocorticoids metabolites levels in faecal matter as validated by Schatz & Palme (2001). Fresh faecal samples were collected each morning following the start of the behavioural data collection, totalling five samples per dog per assessment period. Morning collection ensured that quantity of hormones present would reflect the adrenocortical activity of the previous day (Palme, Touma, Arias, Dominchin, & Lepschy, 2013; Palme, 2005). Immediately after collection, faeces were homogenized in a bag by hand compression, and 10 grams were then transferred to 5ml screwed cap tubes marked with date and the dog's name, and frozen (-20ºC) for later hormonal extraction. To differentiate between faeces of individuals kept in pairs, a non-toxic food colouring dye (Arcolor, blue food colorant) was added to a full spoon of wet dog food and fed to only one of the dogs in the afternoon before the morning collection (the other dog got a spoon full of non-dyed wet food). This type of faecal marking has been widely used for several mammals, including canids (Fuller, Margulis, & Santymire, 2011).

4.3.3.1 Hormonal extraction

Steroid metabolites were extracted from faeces (FCM) following the guidelines of the protocol proposed by Mostl & Palme (2009). Samples were defrosted and 0.5g of wet faecal material was added to a 15 ml conical falcon tube containing 5 ml of a premixed 80% methanol solution. The solution was then vortexed for 2 min in a hand vortex and then placed in a centrifuge for 15 minutes (2500g). From the centrifuged solution, an aliquot of 1ml of the supernatant was transferred to a 1.5ml Eppendorf tube, lyophilized and then stored until it was sent for further analysis with an enzyme immunoassay.

4.3.3.2 Enzyme Immunoassay (EIA)

Using a high-performance liquid chromatography, the faecal steroid metabolites were characterized. After the chromatography separation, the presence of immunoreactive metabolites of glucocorticoids were determined using a cortisol enzyme immunoassay (EIA). Concentrations of glucocorticoids measured by the EIA were expressed as nano grams per gram of wet faeces (Mostl & Palme, 2009).

5.3.4 Decibel levels

Sound pressure levels were collected using a digital sound level meter (Minipa MSL- 1355). Levels were recorded twice per day, at random 15 minutes intervals. Each interval recorded one sound point every second, totalling 1800 sound pressure points per day. Due to equipment and safety restrictions, it was not possible to collect sound data after the facility was closed (after 6pm or before 7am). Hence, the data used to assess the influence of sound on nocturnal behaviours was an average of the sound points collected during the day (as this would be a reflection of the sound profile the dogs experienced during the day and that could contribute to cumulative stress responses; Hewison, Wright, Zulch, & Ellis, 2014; Kawada, 2011).

4.3.5 Light conditions and temperature

The kennels did not possess any source of artificial lighting that could affect the dogs' sleeping patterns. Therefore, data of sunset, sunrise and day length were

retrieved from the Time and Date AS database (© Time and Date AS 1995-2019, Norway), and used as parameters in the statistical analyses. Additional information on temperature for the observed days was retrieved from the National Institute of Spatial Research of Brazil website (CPTEC/INPE, Brazil).

4.3.4 Statistical analysis

All data were tested for normality using the Anderson-Darling normality test. All statistical tests were considered significant at $p<0.05$. Descriptive statistics of all the analysed metrics were conducted and results are presented as either counts or percentages with standard deviation.

Variation in stress hormone expression, sleep timing and decibels levels between sampled days were analysed using Kruskal-Wallis with Dunn's *post-hoc* tests. Differences on decibels levels between periods of the day was analysed using Mann-Whitney tests. Furthermore, correlation between specific days and environmental metrics were explored using a Spearman's rank correlation (Dytham, 2011).

To understand how environmental conditions (explanatory variables) would affect dogs' behaviours (response variables) multiple linear model tests were constructed (GLMMs, lmer function for data with normal distribution and glmer for non-normal data, both with lme4 package in R; Crawley, 2007). The selection of the reported results of the GLMM was made using the minimum model criteria. Significant levels between expressed behaviours were then found using pair-wise comparison (Crawley, 2007). Due to the metrics differences between variables, data were scaled (Becker et al., 1988). Further details of how the models were constructed can be found in Chapter 3, section 3.3.4.

All statistical analyses were carried out using Studio R (RStudio team, 2019).

4.4 Results

4.4.1 Characteristics' of dogs' environment

4.4.1.1 Light and temperature

Luminous emittance in the kennels followed natural light/dark cycles. Throughout the data collection, the average day had 12:46'±00:36' hours of light, sunrise occurred at 05:18h and sunset at 18:05h. The difference between the longest day and the shortest day was 02:14:16 hours, which meant an increase of 16.7% of daylight time.

Temperature varied between 14.8ºC and 25ºC. A variation of six degrees occurred between the analysed months, but temperature changes were observed mostly at the beginning and at the end of the day. Average temperature at midday was 19.9ºC ± 1.12ºC.

4.4.1.2 Sound

The mean sound pressure in the kennels was 63.9 ± 7.0 decibels with mornings being louder than afternoons (Mann-Whitney, $U = 160200$, $p < 0.0001$). A Kruskal-Wallis test indicated the average levels of sound were significantly different between the days of the week ($p < 0.05$), although no significance was found in the *post-hoc* test. While analysing the residuals, Tuesday and Wednesday had many outlier values and where removed from the analysis. Significant levels were still found after removing outliers ($f = 2.95$, $df = 3$, $p<0.05$) and the *post-hoc* test showed Thursdays to be the loudest day at the kennels with a mean value of 64.8 ± 5.9 decibels.

4.4.1.3 Hormonal Profile

Glucocorticoid metabolites (GCM) levels varied between males (Figure 19) and females (Figure 20), with males having higher expression of cortisol than females (Table 8, Figure 21); age did not affected cortisol levels measured ($p>0.05$). Results of the GLMM from the above-mentioned variables are summarized in Table 8. No significant differences between individuals were found ($p>0.05$).

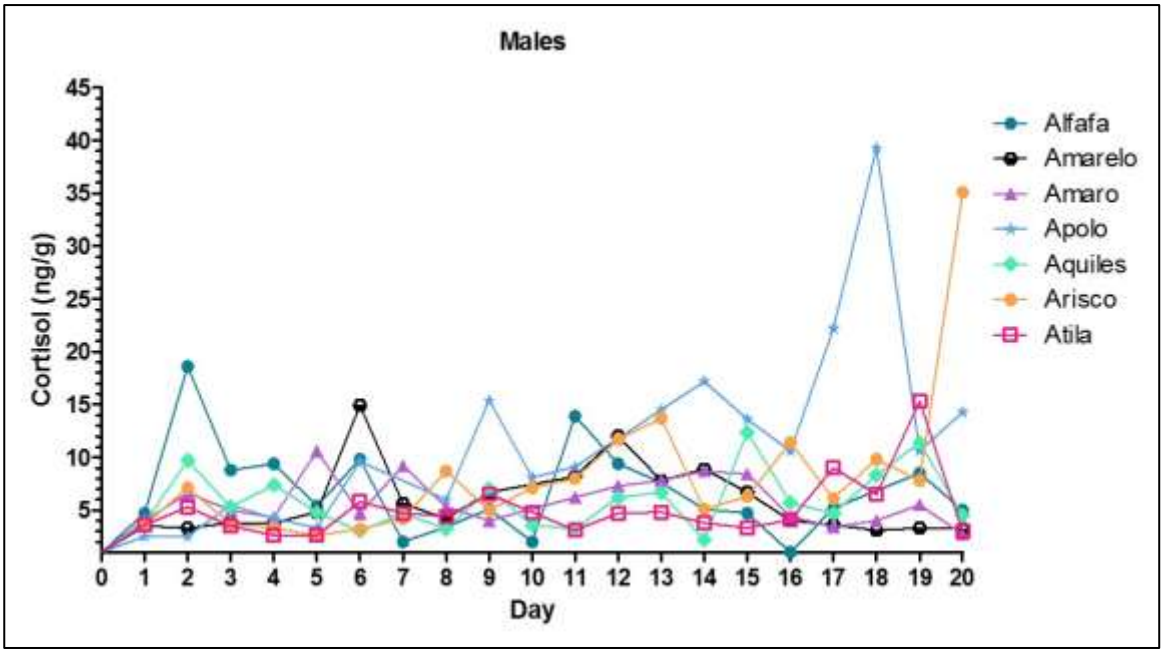

Figure 19. Individual variation of glucocorticoids metabolites (GCM) registered in the male laboratory dogs.

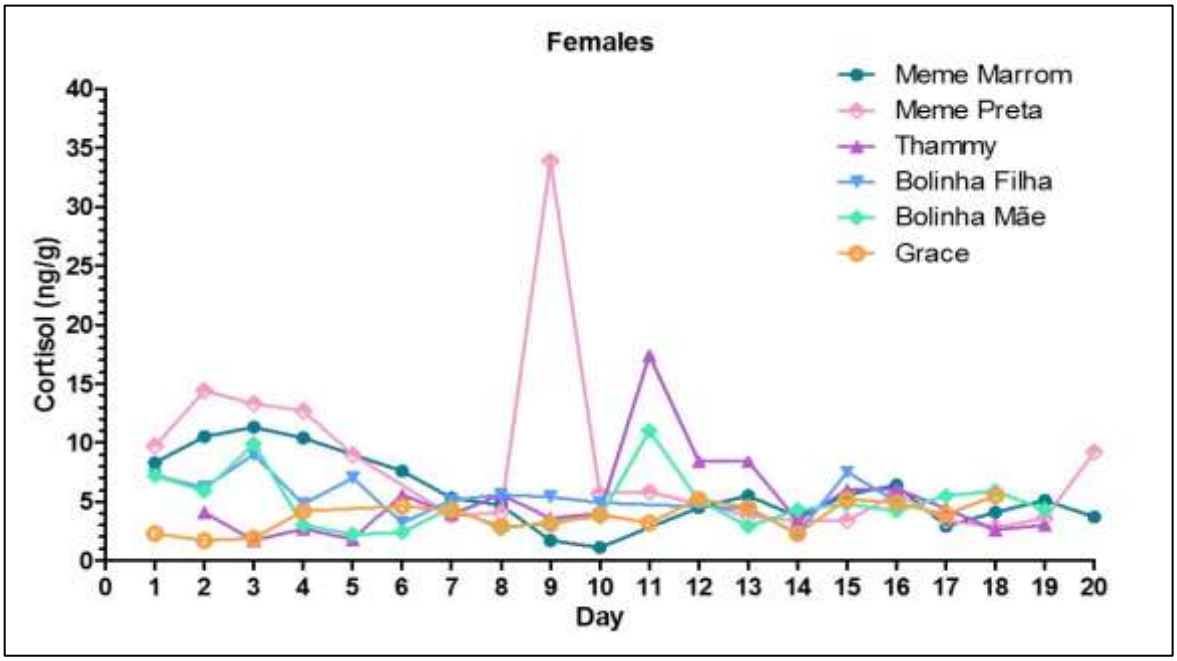

Figure 20. Individual variation of glucocorticoids metabolites (GCM) registered in the female laboratory dogs.

Table 8. Linear Mixed Effects Models results for the optimal models describing the relationship between age, sex, and cortisol as a predictor variable for dogs' stress hormone responses.

Explanatory variable	Parameters	Estimate ± SE	Coefficient value	p	Error distribution
Cortisol	Intercept	6.6165 ± 2.3739	2.787	0.005	Gaussian
	Age	0.0850 ± 0.3607	0.235	ns	
	Sex[a]	1.1005 ± 1.6115	0.682	0.049	

[a] Male is the reference group.

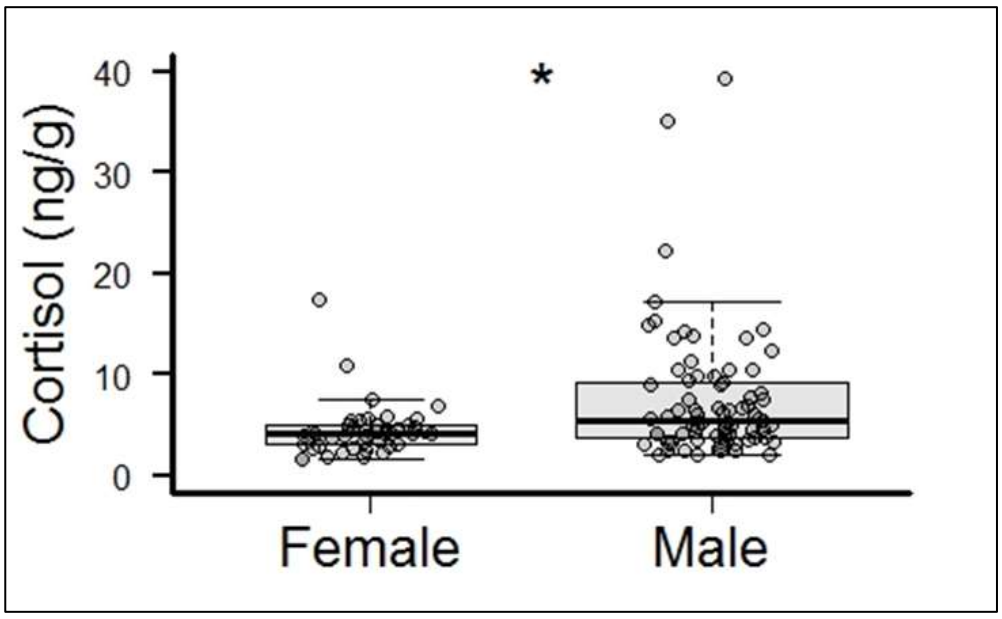

Figure 21. Glucocorticoids metabolites (GCM) levels for female and male dogs. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. * Indicates significant difference at p<0.05.

4.4.2. Environmental effects on sleep and other dog behaviours

4.4.2.1 Light and Temperature

Variation of light had a direct effect on the dogs´ sleeping patterns. Increased day length was associated with increasing number of sleeping bouts in the dogs, while later sunsets (shorter nights) diminished the number of bouts. No effect was found for sunrise. Latency to first sleep did not change with any of the measured

variables, but the awakening time of the dogs did vary with sunrise and sunset. Dogs woke up closer to the kennel staff´s starting time with later sunsets and later sunrises (Table 9).

Similarly, the amount of cortisol found in the dogs' faeces varied according to day length: a longer day was associated with less cortisol in the samples (**Table 9**).

Table 9. GLMM results for the optimal models describing the effects of environmental conditions on sleep related behaviours and physiological components in laboratory dogs

Condition	Independent variable	Estimate ± SE	Coefficient value	p	Error distribution
Sunrise	Sleep bouts	-0.0529 ± 0.0099	-5.333	9.68e-08***	Poisson
	Last bout	-0.5644 ± 0.1225	-4.606	4.10e-06***	
Sunset	Sleep bouts	-1.2503 ± 0.2311	-5.410	6.29e-08***	Poisson
	Last bout	-0.2728 ± 0.1168	-2.333	1.96-02**	
Day length	Sleep bouts	1.0414 ± 0.2319	4.491	7.39e-06***	Poisson
	Cortisol	-2.8143 ± 0.4165	-6.756	2.19 e-06***	

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

A linear regression (r2=0.13, p<0.05) showed that higher temperatures were associated with significantly shorter sleep duration (Table 10, Figure 22).

Table 10. Linear regression results for the effect of temperature on sleep duration at night for laboratory dogs.

Parameters	Coefficient ± SE	t value	Pr(>|t|)
Intercept	162759 ± 28030	5.807	$3.40e^{-08}$***
X	-6988 ± 1408	-4.962	$1.79e^{-06}$***

***p ≤ 0.001.

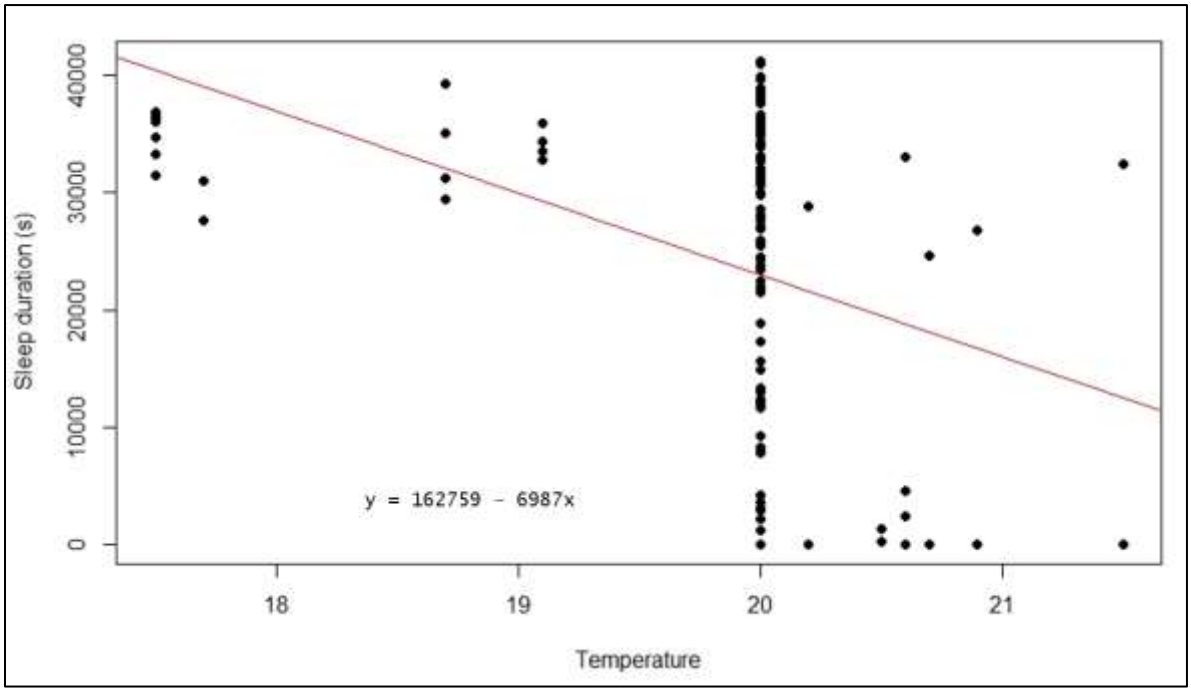

Figure 22. Sleeping duration of kennelled dogs in response to variation of temperature. Trend line based on linear regression result: Sleep duration = 162759 - 6987(temperature), r^2=0.13.

4.4.2.2. Sound levels and stress responses

The variation in sound levels and stress hormones were associated with the expression of measured behaviours in the dogs.

The increase in stress response was associated with the dogs sleeping less and being less inactive during the day by becoming more alert. It was also associated with decreased time spent eating, drinking, playing, performing maintenance and overall locomotion; while vocalizations increased (Table 11). Although no significance effect was found between nocturnal sleep (bouts and duration) and cortisol levels, nor with sound levels, the amount of time spent sleeping during the day and inactivity during the day was significantly affected by these measures (**Table 11**).

A louder environment was associated with the dogs spending less time laydown or sitting inactive, while increasing time spent standing and in locomotion. It was also significantly associated with decreased expression of drinking, eating, exploring and performing maintenance behaviours (Table 11).

Table 11. GLMM results for the optimal models describing the effects of environmental conditions on different behaviours in laboratory dogs. (Family = Poisson).

Behaviour	Response	Estimate ± SE	Coefficient value	p
Alert	Cortisol	-0.0263 ± 0.0126	-2.081	0.037*
	Decibels	0.0049 ± 0.0079	0.630	ns
Drinking	Cortisol	-0.1853 ± 0.0641	-2.890	0.003**
	Decibels	-0.0595 ± 0.0236	-2.522	0.011*
Eating	Cortisol	-0.0333 ± 0.0095	-3.500	4.65e-03***
	Decibels	-0.0337 ± 0.0099	-3.382	7.20e-03***
Laydown	Decibels	-0.0372 ± 0.0023	-15.543	2.16e-16***
Locomotion	Cortisol	-0.0226 ± 0.0045	-5.017	5.24e-07***
	Decibels	0.0161 ± 0.0036	4.469	7.85e-06***
Maintenance	Cortisol	-0.0356 ± 0.0105	-3.386	7.09e-03***
	Decibels	-0.0367 ± 0.0072	-5.100	3.39e-07***
Play	Cortisol	-0.0807 ± 0.0427	-1.886	0.059
Sleeping (day)	Cortisol	-0.0226 ± 0.0070	-3.192	0.001**
Standing	Cortisol	-0.0089 ± 0.0039	-2.276	0.022*
	Decibels	0.0205 ± 0.0032	6.265	3.37e-10***
Vocalizing	Cortisol	0.0261 ± 0.0122	2.627	0.008**

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

The relationship between decibel levels and cortisol levels was explored using correlations, and although no significance was found using total values, breaking the data sets into weekdays established that louder days were associated with increased cortisol levels on the following morning (N= 5, r_s = 0.366, p = 0.026; Figure 23).

Females and males were affected differently by sound levels. For females their stress responses remained constant during the week (N=70 r = 0038, p = 0.775); whereas for males, louder days caused an in increase in stress responses (N = 74, r = 0.366, p = 0.026).

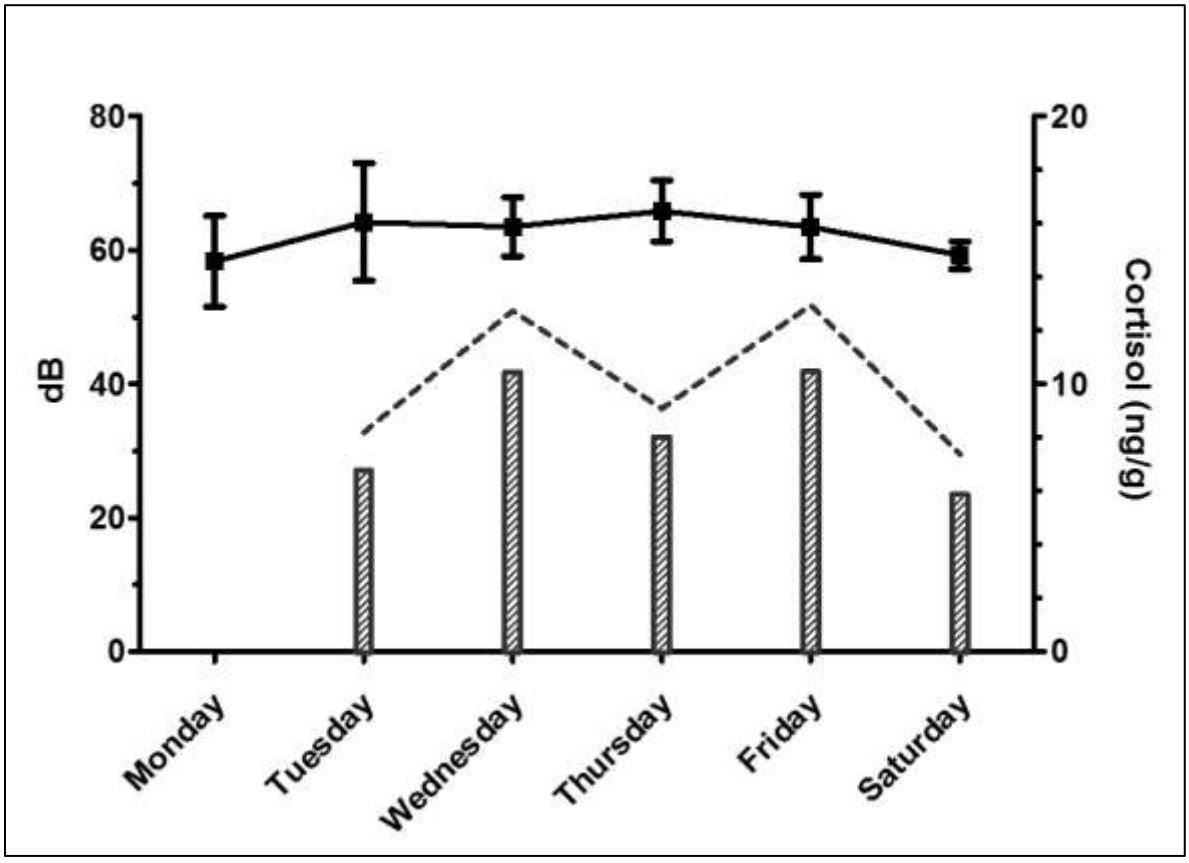

Figure 23. Variation of sound levels (dB) and stress levels (Cortisol) between different days of the week for laboratory dogs. Bars indicate mean levels of GCM, and dashed line shows standard error of the mean. Tick line represents decibels median, whiskers show maximum and minimum values, box represents interquartile ranges.

4.5 Discussion

The dogs in our study were exposed to different environment factors that had a direct impact on their overall behaviour, and that interfered directly in their sleeping patterns. Furthermore, it was possible to deduce that some of the factors had stronger effects on behaviour. Furthermore, that male and females responded differently to these variables (e.g. stress hormone level).

In our investigation, particularly, the variation of stress levels, a physiological response modulated by the environment, and noise were the two main factors of the kennel environment affecting sleep and other behaviours. Stress has been widely studied in humans in relation to sleep, and has found to be a main cause for sleeping disturbances (Jun & Polotsky, 2016; Sadeh et al., 2004; Van Reeth et al., 2000)

As discussed in the previous chapters, our dog population has a compromised sleeping cycle: sleeping less than reported in other studies, not sleeping at all during the day, and having highly fragmented sleep at night. All these factors themselves can be contributing to for stress responses, which can further cause sleep disruption; that is, our dogs are caught in a vicious circle of factors negatively affecting their sleep.

Studies have shown that dogs in shelters (an environment that is similar to the kennels in this study) are exposed to multiple stressors (as reviewed in Polgár, Blackwell, & Rooney, 2019), and generally exhibit higher cortisol levels than pet dogs. In these individuals, the increase in cortisol secretion reportedly triggers: abnormal behaviours, aggression, reduces the immune response, and induces apathy (Protopopova, 2016). Shelters can be considered an over-stimulating environment for dogs, especially in the first days of their arrival, with cortisol levels spiking on these days. Nonetheless, despite levels decreasing after a week, another study found similar levels among long-term sheltered dogs and those in the shelter for only a few days (Titulaer, Blackwell, Mendl, & Casey, 2013).

Even though cortisol levels are expected to stabilize over time this does not necessarily mean an adaptation to the environmental variables present and may become a source of chronic stress(Beerda, Schilder, Van Hooff, De Vries, & Mol, 2000; Part et al., 2014a). Individuals who are trying to cope with prolonged stressors are usually more likely to produce contradictory stressor responses to various environmental stimuli (Chrousos, 2009; Mormède et al., 2007). Whereas, short-duration (acute) stressors induce a very reliable pattern of increase in cortisol levels, prolonged responses (chronic) are more likely to alter the structure of the HPA axis causing a negative feedback of cortisol on brain structures; this response affects the ability of the pituitary gland to recognize its stimulating hormones

(Herman et al., 2016; Mormède et al., 2007). Such patterns are found in humans suffering from Post-traumatic stress disorder (PTSD) or “burn-out” syndrome where the individual's cortisol levels are low despite their inability to cope with the environment (Yehuda, 2009; Yehuda & Seckl, 2011). This could be the case for our dogs; the observed fluctuation of cortisol activity was not consistent across days between individuals. However, the observed cortisol levels were higher than those observed for pet dogs (Hennessy, Davis, Williams, Mellott, & Douglas, 1997) and an increase in stress responses were associated with the increase in variables such as sound levels.

We also found a different effect for males and females, with males having higher cortisol levels than females. Studies investigating stress responses have had mixed results regarding sex differences in dogs. Several found no sex difference for cortisol expression (e.g. Part et al., 2014; Stephen & Ledger, 2006); while others found that females seemed to be more prone to stress when environmental factors such as spatial restriction and social isolation were present (Beerda, Schilder, Van Hooff, De Vries, & Mol, 1999). Moreover, females are more likely to show abnormal behaviours linked to chronic stress - such as tail-chasing - sooner than males (Stephen & Ledger, 2005). Although this matter has not being investigated specifically in dogs, research with humans showed that men tend to have more fight than flight responses in conflicts situations than females, due to stress responses being linked to testosterone levels (Pickering, 2007; Taylor et al., 2000).

Our dogs had restricted space and their social contact is limited. Aside from their regular pairs, they get less interaction with other dogs at play sessions, although those are always with same-sex individuals, for a short period of time (30 minutes) and controlled by human caretakers. The kennels produced a significant amount of noise, especially in the morning, as most of the caretaker activities in and around the kennels was higher earlier in the day. This led to a significant alteration of soundscape with higher levels of decibels recorded in the mornings. The increase in sound also lead to barking, which has a negative nature when the behaviour is expressed excessively, as this makes the environment louder for a long period of

time, and can cause chain reactions which can further increase sound levels (Sales et al., 1997).

All the aforementioned variables seemed to be contributing to producing a situation of chronic stress; however, we do not observe the expression of abnormal behaviours, instead the dogs remained mainly inactive, which could be evidence of learned helplessness and also has been associated with poor affective states (Fureix & Meagher, 2015) . Past studies of kennelled dogs found that when the individuals were less active they had elevated cortisol levels (Hiby, Rooney, & Bradshaw, 2006) . Conversely, some studies pointed that increased stress levels not always change behavioural patterns in dogs (Beerda, Schilder, Van Hooff, De Vries, & Mol, 2000; Hennessy et al., 2001; Rooney, Gaines, & Bradshaw, 2007), hence the importance of using different investigative techniques, such as glucocorticoids measurements, to understand the effects that kennelling has on dogs. Nonetheless, there is an agreement among animal welfare researchers that dogs which remain a long time in shelters are more susceptible to chronic stress and reduced welfare (as reviewed in Polgár et al., 2019).

As sleep has been little investigated with dogs in relation to kennelled environments (Owczarczak-Garstecka & Burman, 2016) further studies are necessary to better comprehend the full effect that long term kennelling has on dogs´ sleep.

Noise was the environmental component that most affected the dogs' behaviour and wellbeing in our study. Noise sensitivity is a common factor affecting dogs' well-being, and noise phobia has found to be a recurrent behavioural problem in dogs (Ballamwar, Bonde, Mangle, & Vyavahare, 2008; Sherman & Mills, 2008; Storengen & Lingaas, 2015). As previously reported, dogs produce stress responses to acute loud noise exposure (Beerda, Schilder, Van Hooff, & De Vries, 1997; Beerda, Schilder, Van Hooff, De Vries, & Mol, 1998; Engeland, Miller, & Gann, 1990).

The sound in the kennels ranged from 46.1 dB to 88.1 dB and the increase of sound above 80 decibels was usually observed when the dogs were barking in situations of high emotional arousal, such as when the staff arrived to clean the

kennels and feed the dogs. Higher levels were also recorded when some of the dogs were out of the kennels while the majority was still housed in their kennels. Moreover, these are all situations that can carry an emotional component; hence the excessive barking could be also a form of frustration.

As described in the literature, frustration arises as an emotional response to an expectation that has not been met, which then generates a mild aggressive response in relation to the delay or absence of a reward (Amsel, 1992).

As any other affective state, frustration has a specific and immediate emotional reaction. Due to its nature, frustration is classified as a negative emotional state, hence being considered a welfare problem as it generates displacement behaviours(Hemsworth et al., 2015; Latham & Mason, 2010). In dogs, several studies have shown that vocalization (barks, growls and whines) is the most common form of manifesting frustration(Jakovcevic, Elgier, Mustaca, & Bentosela, 2013; McPeake, Collins, Zulch, & Mills, 2019).

In humans, the threshold value for sound (noise) annoyance is around 50 decibels (dB), although studies showed that any values over 40dB during the night will compromise human's health (World Health Organization, 2011). Barking sometimes can reach to up 100 dB (Sales et al., 1997), and dogs have a higher (sound frequency) auditory perception than humans do, as they can hear ultrasounds (e.g. machinery and engines), hence the influence of such noise can be much more damaging to them. For humans, any activity performed under sound levels of over 80dB must be mitigated using mufflers and individuals cannot be exposed more than eight hours per day to the source (as specified by UK Control of Noise at Work Regulations 2005).

A range of environments has been investigated relating the effects that common background noise has on dogs. Sources of noise disturbance caused by people voices, machinery and husbandry procedures all produced high level of decibels (above 70dB), which affect dogs' behaviour, physiological parameters and rest/recovery in veterinary clinics (Stellato et al., 2019). In laboratory dogs, the same noise sources altered experiment results (Baldwin, Schwartz, & Hopp, 2007). Shelters/kennels are considered one of the louder environments for dogs to be

housed in, often reaching peaks of 100 dB during the day (Coppola, Enns, & Grandin, 2006; Sales et al., 1997; Scheifele, Martin, Clark, Kemper, & Wells, 2012). In these locations noise produced by visitors, procedures and barking are shown to increase levels of cortisol, expression of abnormal behaviours, compromised immune function and auditory perception in individuals (Kogan, Schoenfeld-Tacher, & Simon, 2012; Protopopova, 2016; Taylor & Mills, 2007).

At our kennels the environmental noise was characterised as being constant and loud, which could contribute to the build-up of stress responses over time (i.e. cumulative stress response; (Beerda et al., 1997; Engeland et al., 1990). This is especially true for barking, when one dog started to bark a chain reaction was observed. Even if only one dog was aroused by some stimuli the others still responded, even those who were in the opposite side of the kennels and would not be able to see or hear the stimuli that triggered the barking..

As expected, the presence of constant barking and other noises during the day were interfering with the dogs' resting, and this was associated with an increased accumulated stress response, as the highest peak of cortisol hormone was observed on the Fridays following the noisiest day of the week. Additionally, males were more affected by this than females. This is most likely due to the fact that males tend to have more fight-or-flight responses to aversive stimuli than females (Taylor et al., 2000), and fight responses can be aggravated by loss of sleep (Hicks et al., 1979).

The behaviour exhibited by our dogs in response to noise corroborates its deleterious effects: dogs became more restless, stopped eating, stopped drinking water, did not sleep and decreased their maintenance behaviour. This together with the elevated cortisol levels, these behavioural changes can be signs of chronic stress (Beerda et al., 1997; Part et al., 2014b). Both light and temperature were also factors, which contributed to elevated stress responses and for changes in the sleeping cycles of the dogs. The mammal circadian clock mediates behaviour and physiology in response to variation in light levels, which also regulates cycles of sleep and activity (Adan et al., 2012; Randler, 2014). It is predicted that environments that are not affected by artificial lights at night would be more suitable for animals, as their sleeping patterns would follow the natural light cycle. However,

as sleep is also regulated homeostatically, sleep timing and duration are also dependent on prior sleeping cycles (Friedman, Bergmann, & Rechtschaffen, 1978; Vyazovskiy & Tobler, 2012). When individuals have normal sleeping patterns disturbed, sleep deprivation motivates the body to seek rest in a different time of the day, and when this is not possible, as it is the case for our dogs, it will disrupt their circadian rhythms (Boivin & Boudreau, 2014; Nunez et al., 2018).

Although our kennels did not have any source of artificial light, we still found a negative relationship between the duration of the day and sunrise and sunset times with sleep in the dogs. The extended light hours in most of the months caused the dogs to sleep less for most of the time studied. The fact that during the day light exposure is constant, and in conjunction to noise and other factors the animals are not able to rest, ended up adding to the increase in stress responses and altered sleeping cycles.

A recent study conducted by de Oliveira and colaborators (2019) found that a nocturnal species of rodent, when exposed to a variation of lights in normal light/dark cycles, also changed their sleeping patterns according to light colour, due to their perception of some wave lengths as day light. A similar effect was also found in birds that can change rhythms based on the spectral composition of sunlight when light intensity is not a reliable environmental cue (Pohl, 1999). Conversely, humans that live in Arctic latitudes show enhanced mood disorders and delayed sleeping time during the dark months (Friborg, Rosenvinge, Wynn, & Gradisar, 2014). Hence, it is possible that natural light can affect behaviour in ways that are not predictable with normal activity cycles. This needs to be explored further.

The same impact was found for temperature, dogs slept less when temperature increased. In endotherms (including humans), sleeping patterns can be disturbed when individuals are exposed to temperature exceeding an individual's thermoneutral zone. The combination of shifts in seasonal temperature and light levels have been shown to cause major behavioural changes in other mammal species such as the Arabian oryx (*Oryx leucoryx*) and the Javan slow Loris (*Nycticebus javanicus)*. The Oryx becomes more inactive during the summer when temperature is higher and expresses an earlier activity onset in winter (Davimes et

al., 2018), while lorises have longer sleeping bouts with increases in temperature (Reinhardt et al., 2019)

The layout of the kennels allowed the dogs to be exposed to the weather as the ceiling only covers part of the kennel and the walls did not reach the ceiling. This meant that sunlight reached most of the space, increasing light exposure and temperature, but it also meant that on colder days, or when it rains, the temperature dropped significantly. Another feature of kennels that contributed to these environmental factors having a great effect on dogs was the fact that the kennels had no insulation on the floor or any type of bed or housing. Hence, when it rained, the floor remained wet and the animals slept on the wet floor. Furthermore, if the position of the sun was hitting a specific angle, there was not enough shade for the dogs to hide in.

Several studies on pen design and welfare have shown that this is one of the most important criteria for dog welfare, and good practices in shelter/kennels should consider both thermal and physical comfort (Hubrecht, 1993; Scullion Hall, Robinson, Finch, & Buchanan-Smith, 2017). Factors such as visibility, choice of resting place, size, easy partitioning of dogs, appropriate shelter (if animals are exposed to weather conditions), insulation, enrichment, and noise-reducing material are crucial to dogs' quality of life and have a direct impact on dogs behavioural and physiological responses (Coppola et al., 2006; Hubrecht, 1993; Normando, Contiero, Marchesini, & Ricci, 2014; Protopopova, 2016; Pullen, Merrill, & Bradshaw, 2010; Scullion Hall et al., 2017; K. D. Taylor & Mills, 2007a)

The females in our study had lower stress responses, perhaps due to the kennel layout in their quarters being different from the males. By having a small room in the back of their kennels, they were provided with more shade, shelter and had a quieter space.

4.6 Conclusion

Environmental factors played a decisive role in sleeping behaviour, stress responses and general welfare of the studied dogs.

Dogs were highly impacted by noise, which seemed to increase during the week and generate accumulative stress responses. The constant present of noise during the day also contributed to reduced time spent sleeping and made the dogs more alert (which again implies a physiological response associated with stress). Males were also more impacted by noise than females. Noise exposure further affected other behaviours that decreased expression whenever the environment became louder.

Light and temperature also affected dogs sleeping patterns. Sleep onset and duration were both affected by increases in temperature and in light exposure. Although the exposure to natural lighting conditions is beneficial for the sleeping cycle, the effect of light in addition to other variables, such as sound and temperature, could be having a negative impact on the dogs.

Furthermore, the kennel design is inappropriate for ensure minimal comfort for the dogs, and the absence of proper insulation, shelter and furniture are affecting the dogs welfare.

Improvements in the kennels are needed to try to mitigate such issues. The presence of barriers to block street noise, shelters for males and females that would improve light and temperature conditions, as well as bedding/flooring for thermal comfort and the habituation of individuals to people and husbandry practices could all contribute to improve the sleep quality of the dogs and their welfare.

Chapter 5. Using wearable technology to measure sleep behaviour and general physiology of laboratory dogs

5.1 Introduction

Sleep is a fundamental physiological process across terrestrial mammals, being an intrinsic part of the homeostatic process and an invaluable behaviour that cannot be eliminated without deleterious consequences to organisms (Cirelli & Tononi, 2008; Orzeł-Gryglewska, 2010; Randler, 2014; Siegel, 2005). Sleep also is a behaviour that has similar behavioural and physiological components across different mammal species (Harold Zepelin et al., 2005). Despite this, different species have different characteristics as to how sleep behaviour occurs (e.g. number of bouts, period of the day), but all species have similar sleeping cycles, starting with slow-wave sleep followed by REM-sleep (Rapid Eye Movement) and then, wakefulness (Lesku et al., 2006; Harold Zepelin et al., 2005).

Sleep is directly affected by the environment and carries an important emotional component, since events experienced during awakening are directly connected with subsequent quality of sleep (Langford & Cockram, 2010; Tougeron & Abram, 2017). Furthermore, studies have shown that sleep quality and quantity is affected by both acute and chronic stress and that lack of sleep is a major stressor in itself (Buckley & Schatzberg, 2005; Jun & Polotsky, 2016).

All these characteristics indicate that sleep can be a reliable animal welfare indicator, although, so far, little research has been conducted to use sleep as a measure, most likely due to the difficulties to measure such behaviour (Lesku, Roth, Rattenborg, Amlaner, & Lima, 2009).

The gold standard method to measure sleep is the use of EEG recordings, which involves the use of electrodes to measure brain waves associated with sleep (I. G. Campbell, 2009). In non-human species, most of the time, this means having to have the electrodes surgically implanted with animals under general anaesthesia and a prolonged post-operative recovery period, a process that can have negative impact on animal welfare (Lesku et al., 2009; Scriba et al., 2013).

An alternative to this would be the use of external adhesive electrodes, a method successfully tested with cows (Ternman, Hänninen, Pastell, Agenäs, & Nielsen, 2012) and owls (Scriba et al., 2013), but not without drawbacks. Cows sometimes rubbed off the electrodes and in owls, an attenuation of signal was observed over time, which affected data collection. Other problems associated with this method is that due to the size of the electrode and way the device is attached, it would not be fitted on some species, such as smaller animals (e.g., mice). Furthermore, the cost and particularities (e.g. need for specific positioning) of such equipment could make a study unfeasible depending on the type of animal and the number of subjects being studied.

In the past decade, the use of accelerometers to measure different behaviours, and behavioural problems has become a trend in animal welfare research (Jukan, Masip-Bruin, & Amla, 2017a; Rushen, Chapinal, & De Passillé, 2012; J. C. Whitham & Miller, 2016). Accelerometers are devices that measure the difference in acceleration forces in relation to the earth (Brown, Kays, Wikelski, Wilson, & Klimley, 2013; Graf, Wilson, Qasem, Hackländer, & Rosell, 2015). In behaviour research, when affixed to an animal (on their legs, neck or back), one to three of these sensors are aligned in a way that each one of them identifies acceleration in a single plane (dimension) of movement – surge, heave and sway (Brown et al., 2012; Wilson et al., 2014, Figure 25). The three sensors working together can represent in real time three-dimensional movement, which gives us precise changes in behaviour (Graf et al., 2015; Jukan et al., 2017a).

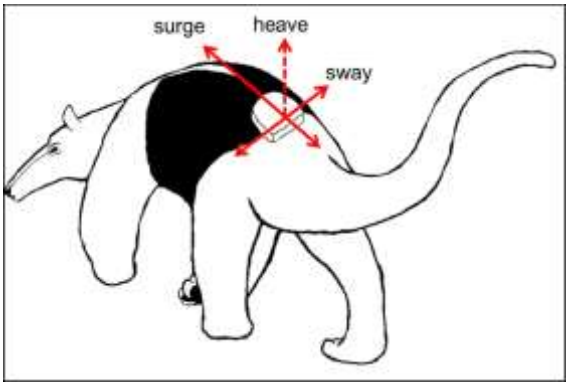

Figure 24. Representation of how a tri-axial accelerometer works on a terrestrial mammal (*Tamandua mexicana*). Extracted from: Brown et al., 2012.

The use of an accelerometer-based technology to measure behaviour has been validated with livestock (Piccione, Giannetto, Casella, & Caola, 2010; Rushen et al., 2012), pets (Clarke & Fraser, 2016; Olsen, Evans, & Duerr, 2016; Piccione et al., 2014), captive and free-ranging wild animals (Lush, Ellwood, Markham, Ward, & Wheeler, 2016; Shepard et al., 2010; J. C. Whitham & Miller, 2016). Accelerometers have been used for differences purposes such as monitoring body movement, postures, reproduction, activity budgets, stress levels, inactivity/rest and most recently sleep (Lush et al., 2016; Nam, Kim, & Lee, 2016; Rushen et al., 2012; Shepard et al., 2010).

Although accelerometer data alone cannot discriminate between sleep phases, the association of this method with other remote assessed physiological measures such as heart rate or temperature (Signer et al., 2010; Williamson et al., 2016) could make this technology a reliable tool to assess sleep quantity and quality (e.g. fragmentation or reduced sleeping times). Another option would be measuring specific behavioural events that happened during sleep; for example, cows, giraffes, elephants and horses all need to lay down in lateral recumbency to achieve REM sleep (**Horse:** DuBois, Zakrajsek, Haley, & Merkies, 2014; **cattle:** Klefot et al., 2016; **elephants:** Schiffmann et al., 2018; **giraffe:** Tobler & Schwierin, 1996).

In dogs, our model species, the use of an accelerometer-based technology has been adapted in the form of wearable collars that can track dogs' activities, behaviour patterns and even stress related responses (Clarke & Fraser, 2016; Olsen et al., 2016; Ortmeyer, Robey, & McDonald, 2018; Weiss, Nathan, Kropp, & Lockhart, 2013; Brian M. Zanghi et al., 2013c). Not only being used for scientific research, these type of collars were further developed into a commercial product that owners can acquire for their pet and veterinarians can use to monitor their patients (Belda, Enomoto, Case, & Lascelles, 2018a).

Nonetheless, despite the different applicability that the collars have, only a few studies have evaluated the precision of accelerometers to predict rest. Whilst the results are promising in relation to head posture and low body movement, further work is still necessary to be able to identify the quality of the rest an animal achieves as monitored by these devices (Clarke & Fraser, 2016; Ladha & Hoffman, 2018a). The downside associated with most of these collars is the absence of further

sensors that can gather physiological measures to be compared to the behaviour recorded by the accelerometer and which could provide further information on more difficult states to evaluate, such as sleep. However, there is the PetPace™ collar (PetPace, LLC, Burlington, MA), which is currently the only commercial wearable sensor that uses a tri-axial accelerometer to measure dogs' activity, while simultaneously collecting, through an acoustics sensor, physiological measures such as respiration rate and heart rate (Belda et al., 2018a).

The aim of this chapter was to evaluate the efficiency of the aforementioned collar in assessing dogs' activity and physiology in an attempt to verify sleep quality and provide indications of the welfare of laboratory dogs.

5.2 Aim

To test the accuracy of a smart collar device to monitor sleep (and physiology in dogs) and, thereby, determine if this device would be a useful tool to investigate sleep quality. This aim is derived from the need to measure sleep using methods that are non-invasive in nature.

5.3 Methods

5.3.1 Study location and subjects

To verify the accuracy of a smart collar to measure sleep quality in dogs, this study was conducted simultaneously with the video-based behavioural observations of dog behaviour described in Chapter 3. Thus, the study location and individuals used in the present study were identical.

5.3.2 Remote sensing of behaviour and physiology

To acquire behaviour and physiological data from the dogs the study used a smart-sensing collar from the brand PetPace™. PetPace™ (PetPace, LLC, Burlington, MA; Figure 26) is a non-invasive wireless collar that continuously collects a dog's vital signs and behaviour patterns, and then transmits the data to

an online base using a gateway connected to the local internet by an ethernet cable on a modem at the animal holding facility.

The collars continuously monitored the dog's activity and body posture through a tri-axial accelerometer and measured heart rate, respiration rate, and heart-rate variation using an acoustic sensor. The device is made from a hard-plastic casing, measuring 40 x 35 x 15mm and weighting 43 grams, attached to an adjustable strap. Collars were fastened on each dog such that the activity monitor was located ventrally on dog´s neck. Each dog had a different collar size (S, M, L) based on the dog's weight, as instructed by the manufacturer (Figure 26).

Figure 25. A) PetPace collar and Gateway. Photo: PetPace website. B) Female dog wearing the PetPace collar during data collection at the kennels of the Centre for Animal Science, Federal University of Ouro Preto, Minas Gerais, Brazil.

5.3.3 Data Collection

In total, eight different collars were used for data collection. Using the Pet Pace website (which is used to manage the collars), it was possible to create individual profiles for each dog and to assign a specific collar to the individuals. This also enabled us to switch collars between animals without losing data. In order to monitor the dogs, the collar was turned on in the laboratory while in range of the gateway, to signal to the database that a dog was being monitored. Once the collar showed as active in the dogs' profile, the collar was then taken to the kennels and fitted to a specific dog. Furthermore, collars were removed once a day and taken

to their internet gateway and stored data were downloaded. This process took no longer than 30 minutes and data from this period were discarded from the analyses. At the end of each week, the data were downloaded from the online database to a Microsoft Excel spreadsheet.

Dogs were assessed from Monday morning until Saturday morning from October 2017 to May 2018, totalling 390 days of assessment.

Aside from the morning intervals to download the data, individuals wore the collars constantly throughout this period. Data extracted from the dogs were summarized in csv files downloaded from the PetPace online database.

For the physiological data, the collar was set to register pulse (HR) and respiration (RR) every 15 minutes, and results were reported as beats per minute and breaths per minute at every point measured (Figure 26).

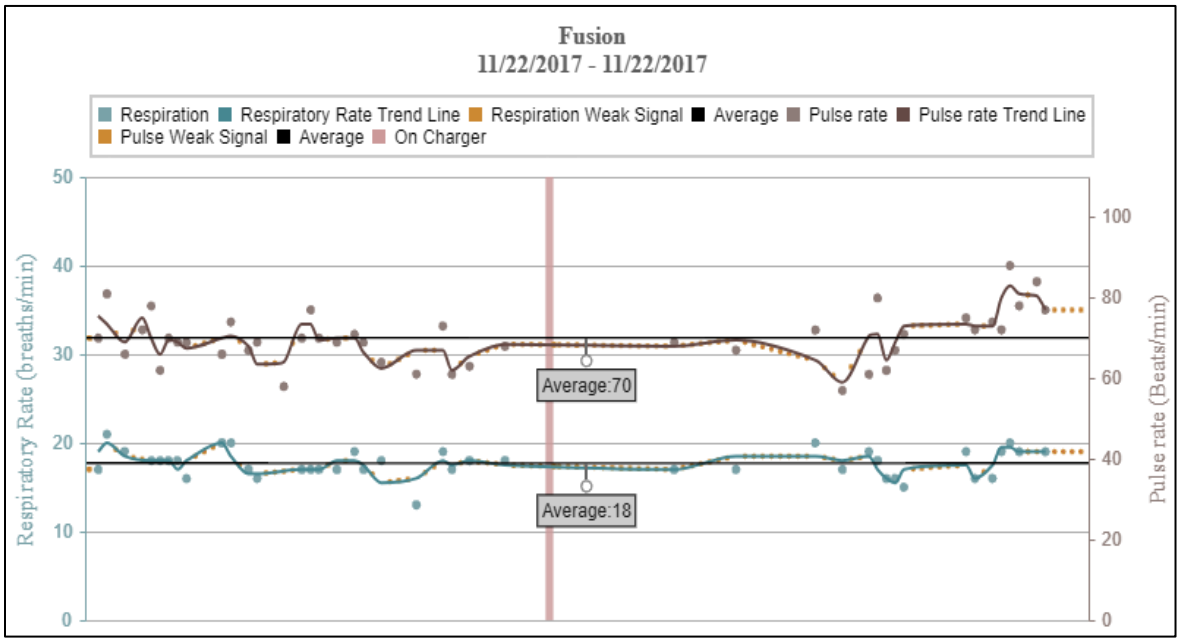

Figure 26. Example of physiological data acquired by remote sensing using the PetPace™ collar on an adult dog. Summary of data over a 24-hour period.

For activity, the accelerometer registered changes in position every second and reported a score with maximum activity every 2-3 minutes (this feature is not adjustable), classifying the activity of the dog as: rest, low, medium, or high (Figure

26). The threshold for activity (between different categories) is specified by an algorithm, which is not disclosed by the company. Additionally, the collar allocated the different measured points into seven behaviours categories: eat, stand, sit, lying right, lying left, lying sternaly, and lying back, which were reported in the file as duration in seconds (Figure 28).

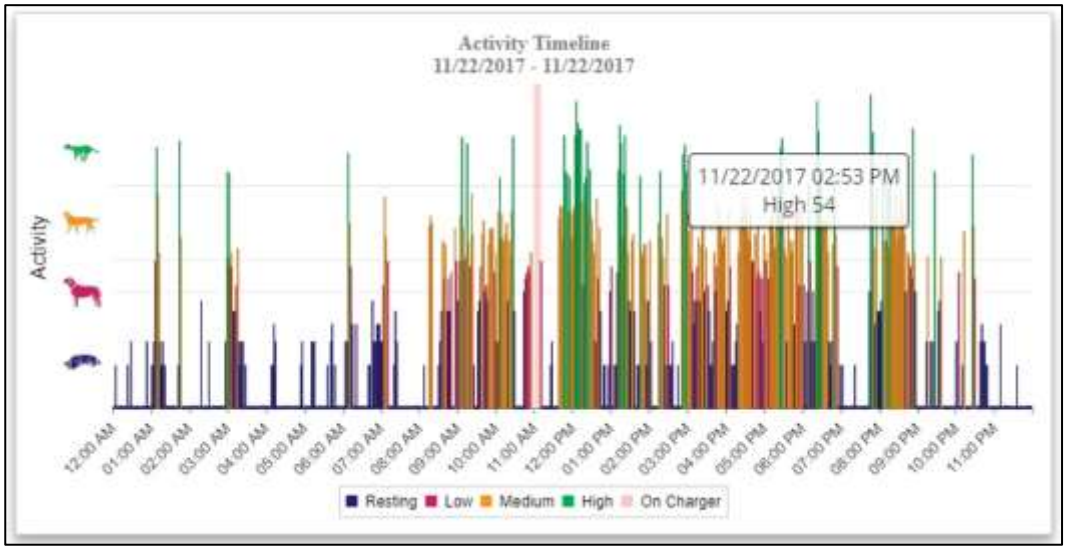

Figure 27. Example of the activity data collected by remote sensing using the PetPace collar on an adult dog. Activity divided automatically into four categories: rest, low, medium and high. Summary of data over a 24-hour period.

For sleep, the collar provided information based on the same points threshold system used for activity and generated automatically a sleep score reported as a percentage (e.g. 73% for 22/11/2017). Hence, it was not possible to account for duration of sleep only using the specific parameter, because the average would be the number of points scored as rest and not the duration of the sleep measured. This also made impractical the calculation of sleeping bouts in this chapter.

To circumvent this problem, an index of sleep was created based on the duration of lying (e.g. sternally or left) recorded by the collar, because the different categories were reported by the collar in seconds. For the index, the average of lying behaviour (all positions) recorded between 18:00 and 07:00 was calculated (same period used for continuous observations of sleeping behaviour explained in the previous chapters). Additionally, we calculated the sum of all rest points equal to zero (no activity) for the same period. Then the average duration of lying was divided by the total number of points for the night, which generated an estimation of

duration of sleep per resting point per night. The same approach was used to verify sleep during the day.

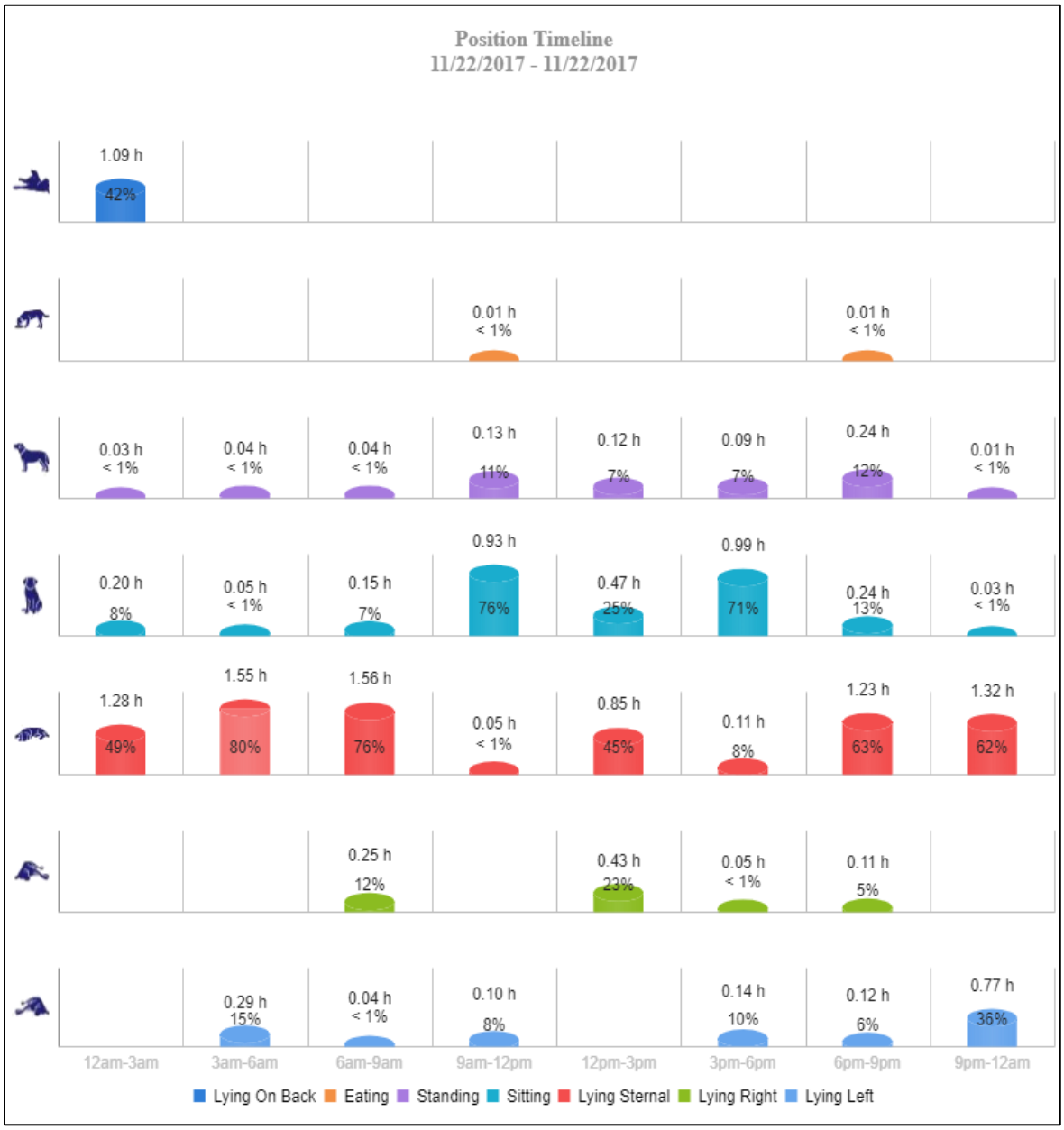

Figure 28. Summary behaviour recorded by the PetPace collar (in seconds). Output generated automatically by their website with hourly percentage and distribution of each one of the seven positions measured during a 24-hour period.

5.3.6 Statistical analysis

All data acquired by the collars were tested for normality using the Anderson-Darling normality test. All statistical tests were considered significant at $p<0.05$. Descriptive statistics of all the analysed metrics were conducted and results are presented as either counts or percentages with standard deviations.

The relationship between the different categories of activity was verified using a Wilcoxon ranked test, in order to decide which groups were significantly different from each other. To verify difference in behavioural expression between categories of behaviour, a Friedmann test with Dunn´s *post-hoc* test was used (Dytham, 2011). Spearman rank correlations were used to verify relationship between different physiological measures (Dytham, 2011).

l Multiple linear models (GLMM, lmer function for data with normal distribution with lme4 package in R; Crawley, 2007) were constructed to understand the relationship between different categories of behaviour (explanatory variables) and physiological variables (response variables). Further GLMM models analysed the relationship between environmental variables such as cortisol, period of the day and decibel levels (explanatory variables) and the changes in physiological response (response variables). The best fit models were selected using the minimum model criteria and significant levels were found using pair-wise comparison (Crawley, 2007). Further details of how the models were constructed can be found in Chapter 3, section 3.3.4. Data were scaled to reduce statistical errors (Becker et al., 1988).

Lastly, in order to verify the efficiency of the measures acquired by the collar against the metrics recorded by the video observations (described in Chapter 3), behaviours were separated in three large categories: Activity, Inactivity and Sleep. From the baseline data, percentages were estimated for each category by method, as well total values for Day vs Night observations and results compared using Wilcoxon ranked test for paired measures (Dytham, 2011).

Additionally, to verify if the point-based system of the collar would also be a good metric to compared against the behavioural observations, two categories were created based on the most expressed behaviour for each period of observations: total activity for diurnal recordings and total sleep for night recordings. Once again,

variables were tested against each other using Wilcoxon ranked test for paired measures (Dytham, 2011).

All statistical analyses were carried out using Studio R (RStudio team, 2019).

5.4 Results

5.4.1 Characteristics of behaviour and physiology as collected by PetPace collars

Dogs wore collars and were monitored throughout 390 days and nights, generating 488800 points of activity and physiological data acquired by the collar. Data loss due to technical difficulties, misplacement or malfunction of equipment was found in 12% of all recorded data.

Overall, activity levels classified as rest were the highest recorded by the collar (66.2% ± 2.6%), followed by medium (18.9% ± 19%), low (9.5% ± 1.6%) and high (5.4% ± 2.7%) activity points (Table 12, Figure 29). Higher levels of activity were most recorded at 09:00 hrs and at 18:00hrs. Activity during the day varied, but a steady decrease in activity was observed from 18:00hrs onwards and rest (sleep) was recorded at similar levels from 22:00 hrs until 07:00hrs in the following morning.

Variation in activity levels were significantly different between periods of the day, as evaluated by the Wilcoxon test. Rest was recorded most at night (W= -151.0, p = 0.018), while medium (W=262.0, p=0.007) and low (W= 259.6, p=0.002) activity were most recorded during the day. The expression of high activity did not differ between day and night (Figure 29).

Table 12. Activity points for laboratory dogs as calculated by PetPace collars during the 390 days of data collection.

Activity threshold	Mean ± SD	Minimum	Maximum
Rest	8.8 ± 0.04	0.00	25.21
Low	28.99 ± 1.81	26.51	31.68
Medium	39.11 ± 4.39	32.70	47.96
High	53.71 ± 4.86	48.25	99.96

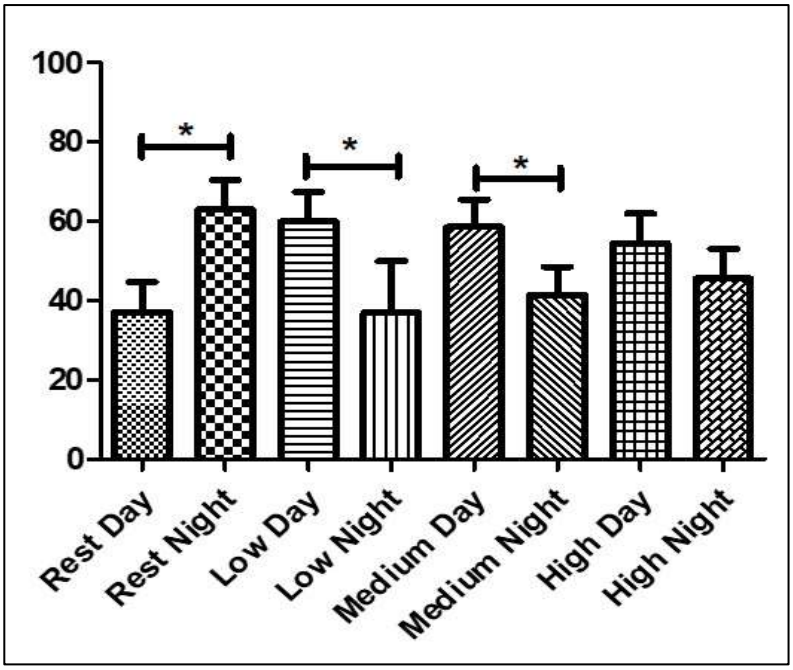

Figure 29. Mean expression of different activity levels (as percentages) of laboratory dogs as measured by PetPace collars over 390 days and nights. Lines above columns with stars show significant differences between categories ($p<0.05$). Error bars show ± standard error of the mean.

Conversely, the different categories of behaviour assigned by the collar also indicated differences in the mean expression of behaviours by the dogs both during the day ($F = 42.00$, $df = 3$, $p <0.0001$; Table 13, Figure 31) and night ($F= 39.94$, $df = 3$, $p< 0.0001$; Table 13, Figure 32). During the day lying sternally (33.2% ± 21.3%) was the most observed behaviour followed by sitting (30.2% ± 20.8%), while at night lying sternally was responsible for almost half of the recordings (43.5% ± 20.3%).

Dogs had a mean heart rate of 69.8 ± 11.6 beats per minute (range 33 – 165 bpm) and a mean respiration rate of 17.2 ± 1.8 breaths per minute (range 11 – 24 breaths). Significant variation between day and night for these variables was only found for respiration, with dogs having higher number of breaths per minute during the day than at night ($F= 68830$, $df = 1$, $p = 0.02$). Heart rate and respiration rate were found to be highly correlated, with increase in heart rate associated with an increase in respiratory rate ($r_s = 0.777$, $p <0.001$).

Individual animals had different frequencies of heart rate and respiratory rate (Table 13). Nonetheless, it was possible to observe that paired animals had similar patterns of variation in HR and RR (Figure 33 to Figure 36). Moreover, females had

higher respiratory rates than males, as verified by a Mann-Whitney test (U= 10080, p<0.01). sex and age did not significantly affect heart rate.

Table 13. Individual characteristics of laboratory dogs' activity, rest and physiological parameters acquired from PetPace collars over 390 nights.

ID	Activity (±SD)[1]		Rest (±SD)[1]		HR (±SD)[2]		RR (±SD)[3]	
	Day	Night	Day	Night	Day	Night	Day	Night
Alfafa	33.2 ± 3.6	21.0 ± 4.4	66.8 ± 8.6.	79.0 ± 7.6	65.7 ± 33.9	63.0 ± 13.9	13.5 ± 6.2	14.8 ± 3.3
Amarelo	51.9 ± 3.2	47.1 ± 3.2	48.1 ± 5.6	52.9 ± 5.6	69.9 ± 6.2	67.6 ± 4.5	17.4 ± 1.2	16.3 ± 0.8
Amaro	36.7 ± 3.4	22.3 ± 3.8	63.3 ± 9.3	77.7 ± 7.7	70.8 ± 23.9	63.8 ± 19.5	16.2 ± 6.5	16.1 ± 5.0
Apolo	44.2 ± 3.5	22.8 ± 3.6	55.8 ± 9.5	77.2 ± 7.7	54.5 ± 38.7	64.7 ± 25.4	12.3 ± 8.1	15.6 ± 6.4
Aquiles	36.4 ± 3.5	22.6 ± 3.8	63.6 ± 9.2	77.4 ± 7.7	52.2 ± 31.4	52.1 ± 26.5	13.1 ± 7.8	12.9 ± 6.5
Arisco	33.0 ± 3.6	20.4 ± 3.4	67.0 ± 8.6	79.6 ± 7.4	44.7 ± 31.6	45.9 ± 28.0	11.3 ± 8.4	12.0 ± 7.4
Atila	45.9 ± 3.8	23.1 ± 4.0	54.1 ± 9.6	76.9 ± 8.0	56.8 ± 33.8	50.9 ± 34.8	12.9 ± 8.1	12.4 ± 8.5
Bolinha Filha	41.9 ± 3.9	13.4 ± 4.9	58.1 ± 9.1	86.6 ± 6.6	62.6 ± 19.4	68.2 ± 14.8	16.2 ± 5.0	17.2 ± 3.7
Bolinha Mae	47.7 ± 3.4	16.8 ± 3.4	52.3 ± 9.3	83.2 ± 5.6	55.4 ± 21.2	52.9 ± 19.7	14.2 ± 6.4	13.7 ± 5.1
Grace	53.5 ± 4.4	39.8 ± 4.3	46.5 ± 5.7	60.2 ± 5.7	67.8 ± 28.2	67.1 ± 22.7	14.8 ± 7.5	15.7 ± 7.2
Meme marrom	49.7 ± 4.0	18.5 ± 4.1	50.3 ± 9.5	81.5 ± 6.9	68.1 ± 27.3	56.6 ± 23.0	15.7 ± 5.8	14.2 ± 6.0
Meme Preta	43.6 ± 3.8	19.3 ± 4.4	56.4 ± 9.7	80.7 ± 6.6	61.3 ± 25.2	60.8 ± 20.7	15.7 ± 6.2	15.5 ± 5.3
Tammy	54.4 ± 3.2	40.9 ± 3.4	45.6 ± 5.8	59.1 ± 5.5	66.9 ± 28.3	65.9 ± 23.3	16.4 ± 6.7	16.4 ± 5.7

[1] Percentage of average points. [2] HR = Heart Rate measured in beats per minute. [3] RR = Respiratory Rate measured in breaths per minute.

Although the collar was able to register data regarding physiology, in some subjects, the collar was observed to have moved and the sensor was displaced from the below the neck position, which caused the HR and RR to be recorded as zero (HR 14.1%, RR 15.6% of data loss due to misplacement).

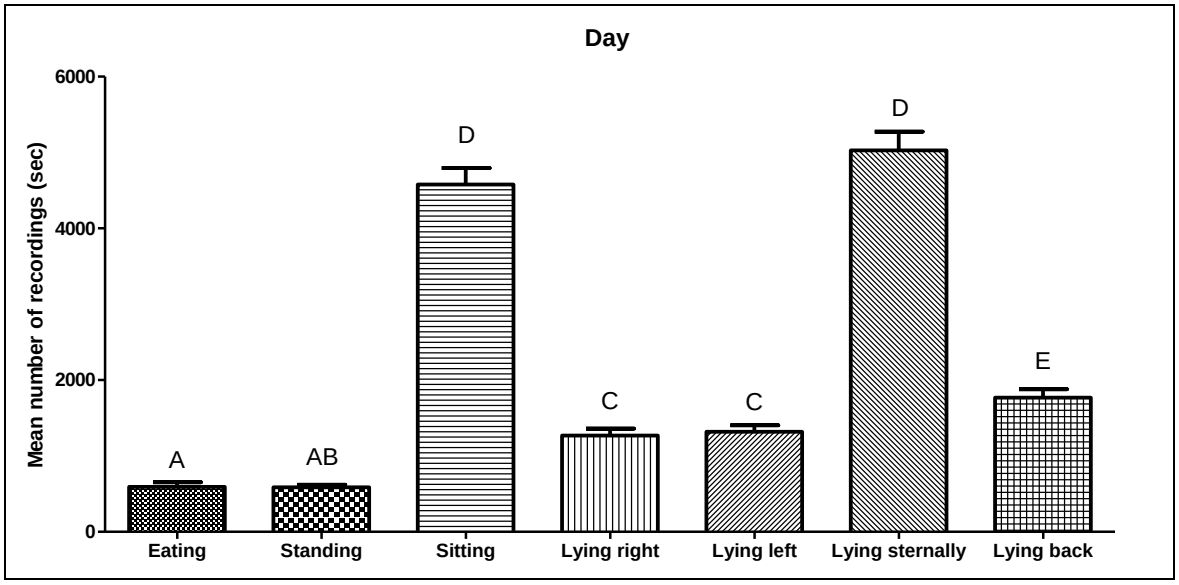

Figure 30. Mean expression of diurnal behaviours of laboratory dogs, as recorded by PetPace collar over 390 days. Capital letters show significant differences ($p<0.05$) between categories. Error bars show ± standard error of the mean.

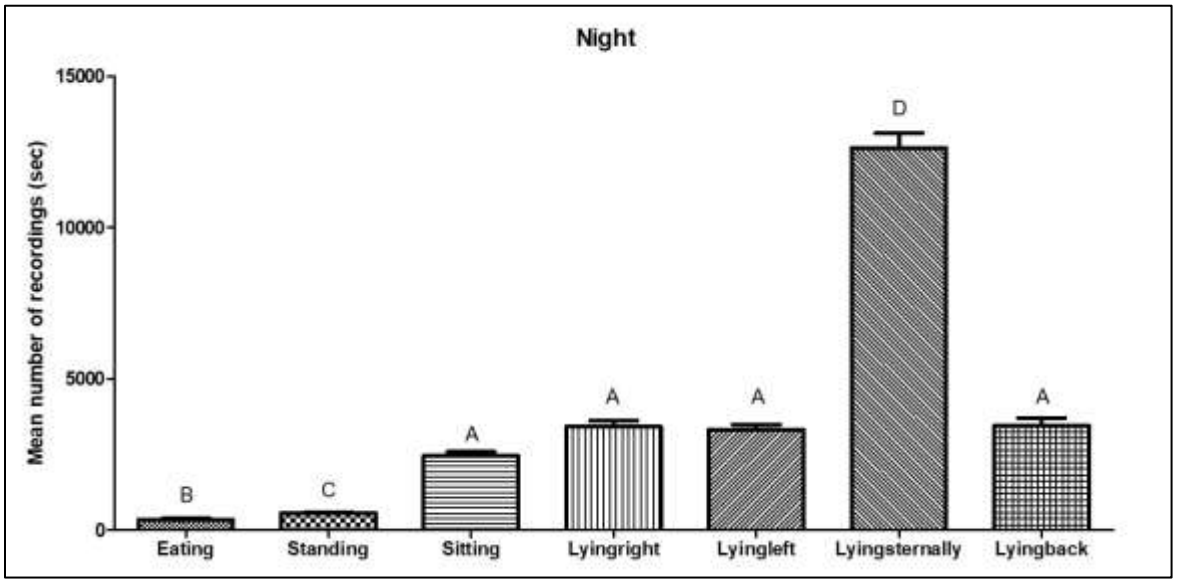

Figure 31. Mean expression of nocturnal behaviours of dogs, as recorded by PetPace collars over 390 days. Capital letters show significant differences ($p<0.05$) between categories. Error bars show ± standard error of the mean.

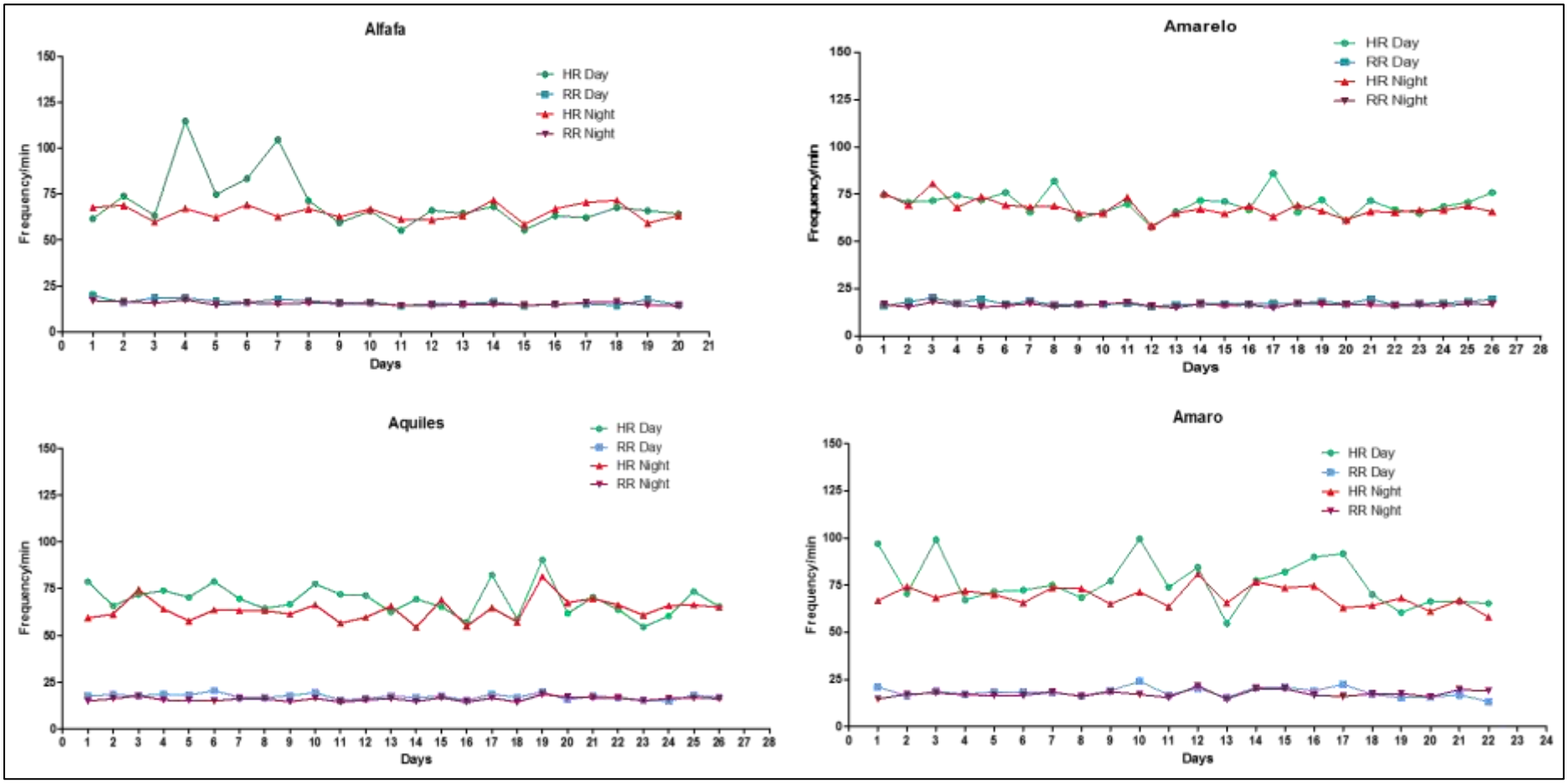

Figure 32. Heart Rate (HR) variation and Respiratory Rate (RR) variation from the PetPace collars during different periods of the day and observed similarities in parameters between paired male dogs (top and bottom subjects, respectively).

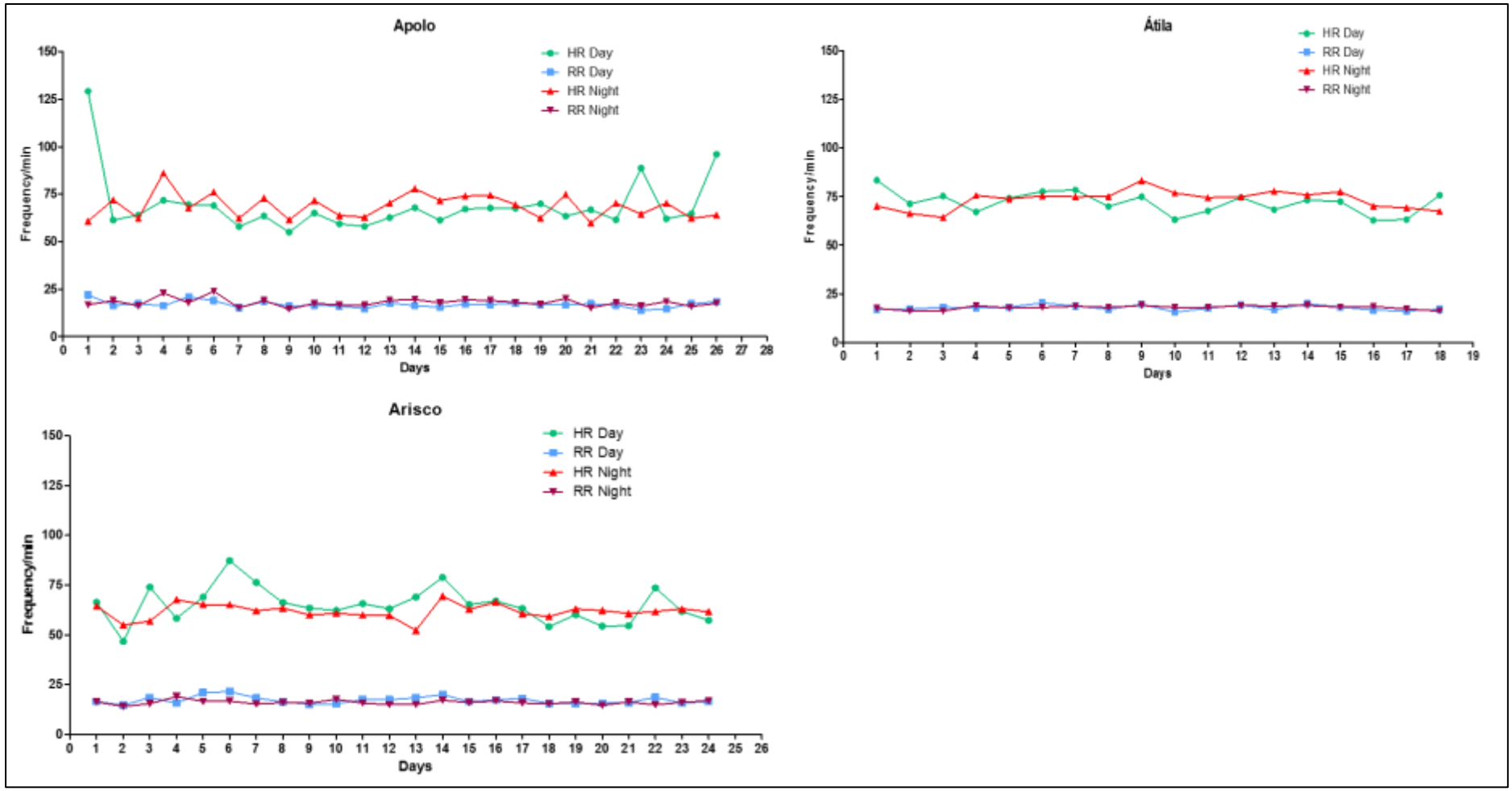

Figure 33. Heart Rate (HR) variation and Respiratory Rate (RR) variation from the PetPace collars during different period of the day and observed similarities in parameters variation between paired male dogs (top and bottom subjects, respectively). Subject Atila was kennelled alone.

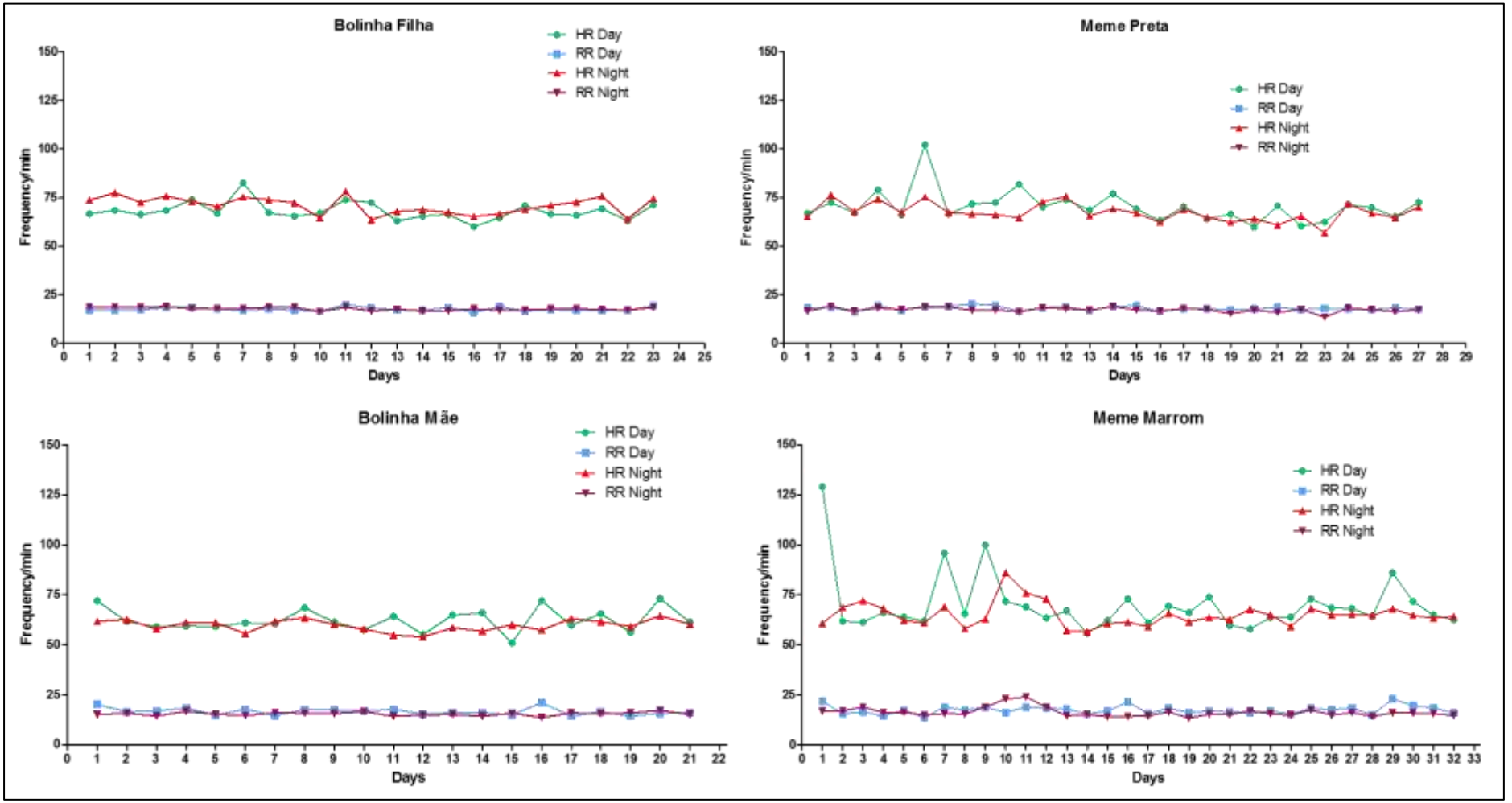

Figure 34. Heart Rate (HR) variation and Respiratory Rate (RR) variation from the PetPace collars during different period of the day and observed similarities in parameters variation between paired female dogs (top and bottom subjects, respectively).

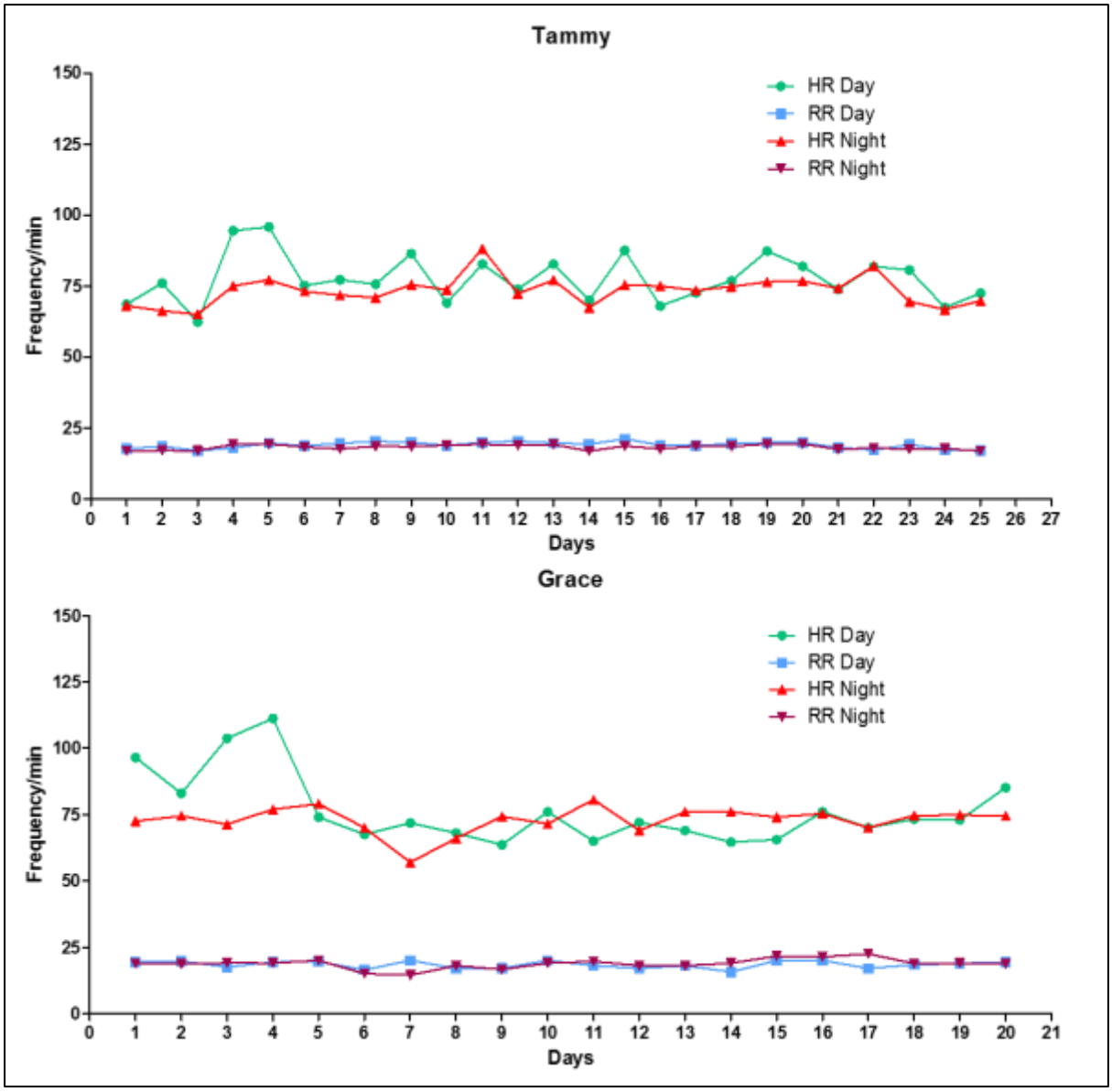

Figure 35. Heart Rate (HR) variation and Respiratory Rate (RR) from the PetPace collars during different period of the day and observed similarities in parameters variation between paired female dogs.

5.4.1.1 Sleep parameters

On average, dogs slept 6.6 ± 1.6 hours (23598 ± 5858 seconds) during the night and 57.9% of the total number of rest points scored as zero occurred between 00:00hrs and 05:00hrs. During the day, sleep was recorded for 0.7 ± 0.4 hours (2545 ± 1631 seconds) and 37.11% of rest points were acquired between 10:00 hrs and 12:00hrs (Table 14). Sleep was significantly different between periods of day, with dogs sleeping mainly at night ($W = -91.0$, $p<0.001$). Sleep was also differently

expressed between individuals: Atila was the dog that slept for the longest (F= 4.999, df = 12, p <0.05; Figure 37). No difference was found when comparing males and females sleep means nor for dogs of different ages.

Table 14. Individual characteristics of sleep from PetPace collars for the laboratory dogs participating in the study over 390 nights.

Males	Sleep (±SD)[1]	Females	Sleep (±SD)[1]
Alfafa	7.3 ± 4.1	Bolinha Filha	6.6 ± 2.7
Amarelo	5.6 ± 3.1	Bolinha Mae	5.9 ± 2.5
Amaro	7.0 ± 2.6	Grace	4.1 ± 2.6
Apolo	6.3 ± 5.3	Meme marrom	7.0 ± 4.4
Aquiles	7.4 ± 4.3	Meme Preta	6.9 ± 3.4
Arisco	6.0 ± 4.1	Tammy	4.3 ± 2.2
Atila	10.7 ± 2.3		

[1]Mean expressed in hours.

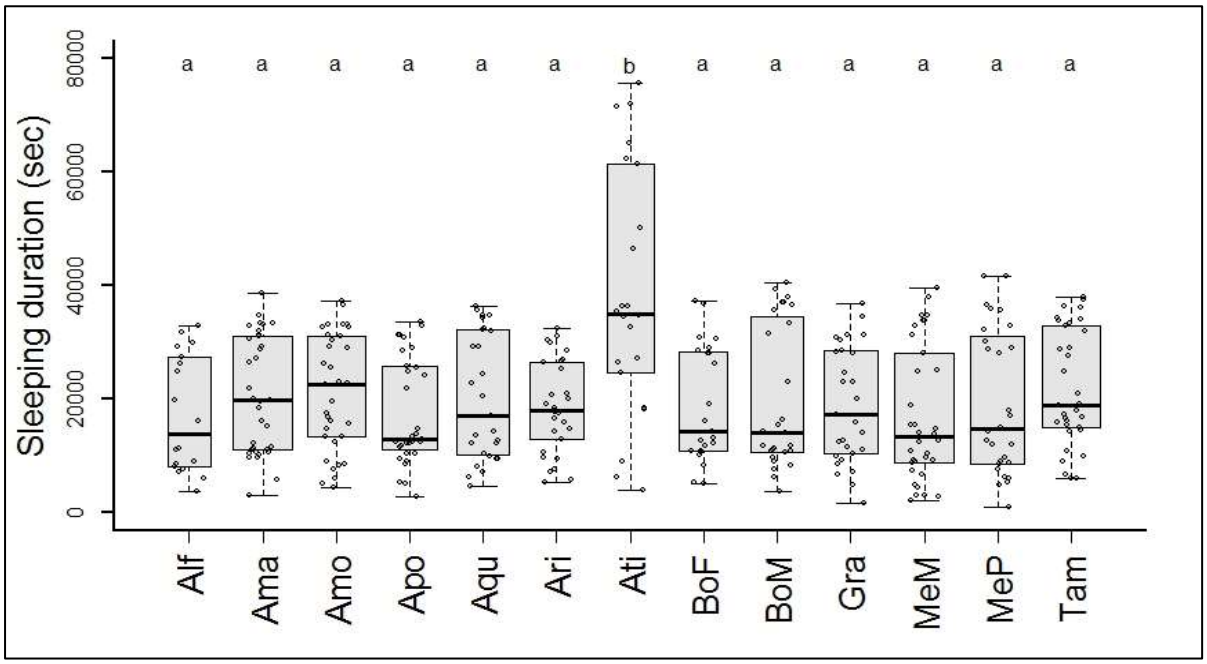

Figure 36. Individual variation on dog sleep as recorded by PetPace collars. Tick line represents median, whiskers show maximum and minimum values, box represents interquartile ranges. * Lower case letters indicate difference at p<0.05.

5.4.2 Relationship between physiology, behaviour and sleep using the metrics acquired by the PetPace collar

Using General Linear Mixed Effect models, the effect of physiological parameters was tested again the behaviours measured with the collar. Complete results are summarized in (Table 15).

Dog activity was higher during the day ($t = -8.687$, $p<0.001$) but decreased with the increases of sleeping duration ($t = -2.382$, $p<0.05$). Higher activity also was correlated with higher pulse rates ($t = 2.418$, $p< 0.05$) and respiratory rate ($t = 2.418$, $p< 0.05$). Older dogs also displayed more activity than younger ones ($t = 3.718$, $p<0.001$).

Sleep was significantly higher during the night ($t = 22.218$, $p<0.001$) and increased with the increase of respiratory rate ($t = 7.762$, $p<0.001$). Dogs also demonstrated less time sitting with longer sleep duration ($t = -4.496$, $p<0.01$); whereas, standing increased in relation to longer sleeping times ($t = 7.927$, $p<0.001$).

An increase in heart rate ($t = 2.919$, $p<0.01$) and respiratory rate ($t = 2.028$, $p<0.05$) were observed whenever dogs spent more time standing; whereas, sitting was positively associated only with higher respiratory rates ($t = 4.693$, $p<0.01$), observed most during daylight hours ($t = -9.225$, $p<0.01$) and in higher frequency in older dogs ($t = 4.740$, $p<0.01$). Lastly, dogs ate more during the day ($t = -3.367$, $p<0.01$) and whenever their heart rate was elevated ($t = 3.512$, $p<0.01$).

Further correlations explored the relationship between physiological variables. The number of sleep bouts calculated from the observations described in Chapter 3 were correlated with the number of heart beats per minute and the number of breaths per minute, but no significant relationship was found.

Cortisol measurements taken from Chapter 5 were also correlated with pulse and respiration, and both physiological variables were associated, although the effect was weak. Heart Rate decreased with increases in cortisol ($r_s = -0.120$, $p<0.01$), and the same effect was found for Respiration Rate ($r_s = -0.125$, $p<0.01$) but again both effects are weak.

Table 15. GLMM results for the optimal models describing the relationships among physiology, behaviour and sleep in laboratory dogs using the metrics from PetPace collars (independent variables). Error family = Gaussian.

Behaviour	Independent variable	Estimate ± SE	Coefficient value	p
Activity	Age	0.5450 ± 0.1466	3.718	2.17e^{-04***}
	Heart Rate	0.0492 ± 0.0203	2.418	0.015*
	PeriodNight	-6.2282 ± 0.7169	-8.687	2.0e^{-16***}
	Respiratory Rate	0.3166 ± 0.083	3.787	1.65e^{-04***}
	Sleeping duration	-0.9009 ± 0.3783	-2.382	0.017*
Eating	Heart Rate	0.0058 ± 0.0016	3.512	4.72e^{-04***}
	PeriodNight	-0.2387 ± 0.070	-3.367	8.02e^{-04***}
Sitting	Age	0.0834 ± 0.0176	4.740	2.58e^{-06***}
	PeriodNight	-0.6184 ± 0.0670	-9.225	< 2e^{-16***}
	Respiratory Rate	0.0298 ± 0.0063	4.693	3.22e^{-06***}
	Sleeping duration	-0.1677 ± 0.0373	-4.496	8.54e^{-15***}
Standing	Heart Rate	0.0074 ± 0.0025	2.919	0.003**
	Respiratory Rate	0.0209 ± 0.010	2.028	0.042*
	Sleeping duration	0.2899 ± 0.0365	7.927	8.54e^{-15***}
Sleeping	PeriodNight	1.1770 ± 0.0531	22.128	< 2e^{-16***}
	Respiratory Rate	0.0398 ± 0.0051	7.762	2.87e^{-14***}

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

5.4.3 Evaluating the efficiency of the PetPace collar against behavioural observations made by a human observer

When evaluating the methods based on behaviours categories, inaccuracy between methods ranged from 0.13% to 59.3% for diurnal observations and 0.1% to 95.9% for nocturnal observations (mean 18.7% ± 11.6% and 19.8% ± 24.5%, respectively) (Table 16.) Significant differences were found between the methods. The collar registered more activity during the day (W = -7986, p<0.0001) and sleep at night (W = - 8065, P<0.0001) , but less activity at night (W = 8333,

P<0.0001) and inactivity during the day (W = 8244, P< 0.0001) than the behavioural observations made from the video recordings (Figure 38).

Table 16. Inter-method differences for categories of behaviour measured in laboratory dogs.

Categories	Behaviour (±SD)[1]	Collar (±SD)[1]	Range[2]	Difference[3]
Activity Day	34.8% ± 15.9%	45.2% ± 15.6%	0.1% – 59.3%	10.5%
Inactivity Day	65.2% ± 15.9%	53.5% ± 16.0%	1.4 – 75.5%	11.6%
Activity Night	25.9% ± 26.5%	11.3% ± 6.0%	0.2% - 92.4%	14.6%
Inactivity Night	9.1% ± 7.1%	9.6% ± 7.6	0.1% - 35.0%	6.4%
Sleep Night	60.0% ± 29.1%	77.8% - 14.6%	1.8% - 97.0%	17.8%

[1]Average observation. [2]Minimum and Maximum values observed. [3]Mean difference for paired observations.

In a similar way, when looking the categories activity and sleep using the collar points threshold, differences were found for activity at night (W = -10850, p <0.0001) and activity during the day (W = 91.00, p = 0.0002) between the methods. On the contrary, no difference was found for the sleep category (Figure 39). Furthermore, when we tested one method against the other using absolute values per animal per category, not only was no difference found for sleep, but also, activity was measured equally between the collar and the observations (Figure 39).

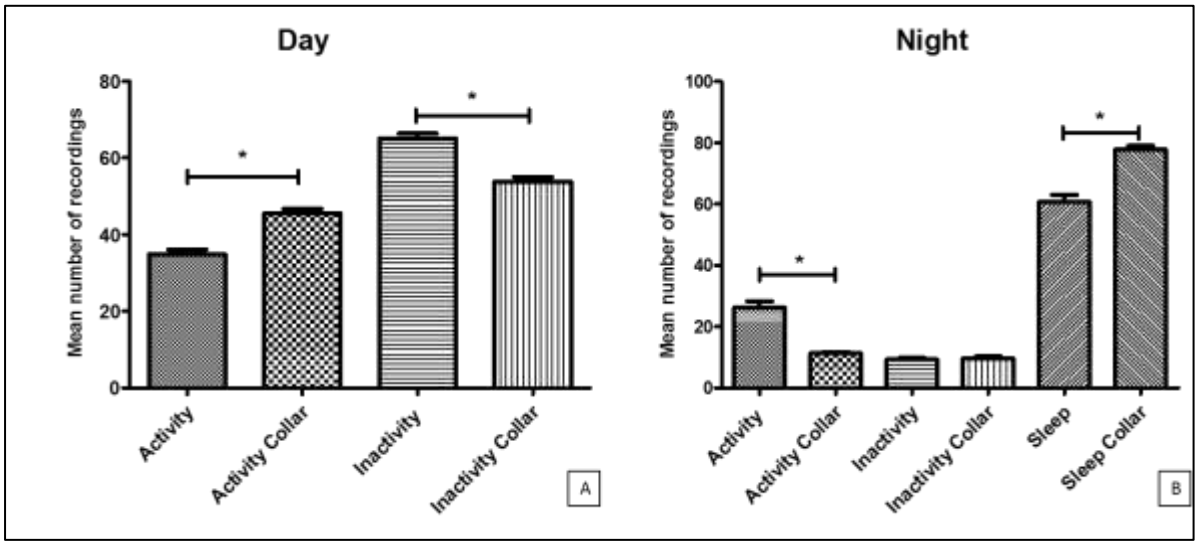

Figure 37. Comparison of behavioural recordings using two different methodologies: the PetPace collar and behavioural observations from videos. A) Mean expression of activity and inactivity during the day as recorded by different methods. B)

Mean expression of activity, inactivity and sleep during the night, as recorded by different methods. Lines above columns with stars show significant differences ($p<0.05$). Error bars show ± standard error of the mean.

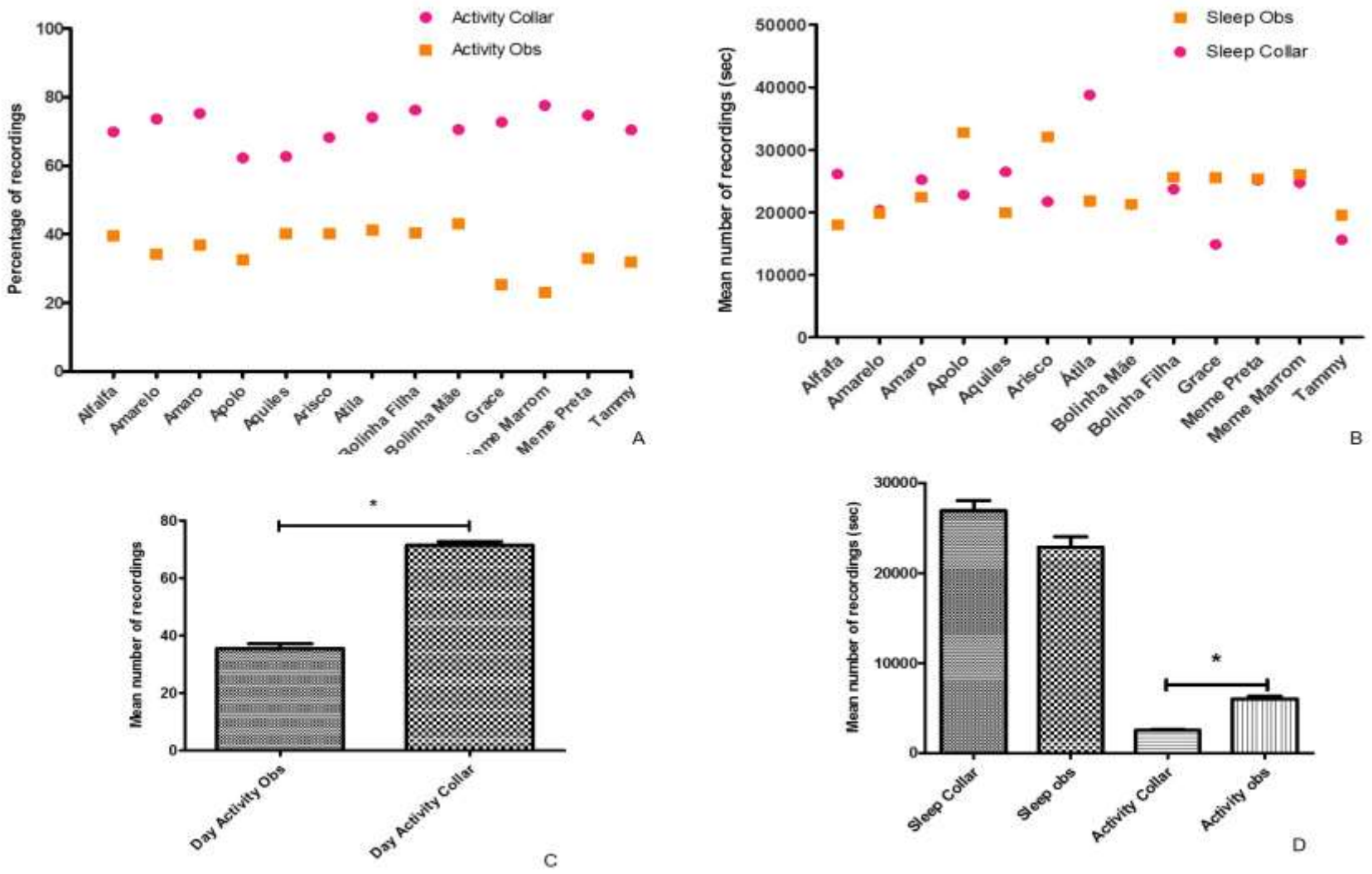

Figure 38. Comparison between automatic results generated by the PetPace collar activity threshold and the metrics recorded using video observations. A) Individual variation of activity as recorded by different methods. B) Individual variation of sleep behaviour as recorded by different methods. C) Mean expression of diurnal activity as recorded by different methods. D) Mean expression of activity and sleep during the night, as recorded by different methods. Lines above columns with stars show significant differences ($p<0.05$). Error bars show ± standard error of the mean.

5.5 Discussion

5.5.1 Characteristics of behaviour and physiology as collected by a wearable technology collar

The objective of this chapter was to evaluate the efficiency of a wearable technology (PetPace) collar in assessing activity, rest and physiological data that combined could provide further information on sleep quality and be used as a tool to measure animal welfare. The results suggested that the collar is a valuable tool when assessing dogs' activity and variation in behaviour, being able to assess dog behavioural budget and monitor behaviour fluctuations along the day, although not without limitations that would be further discussed in this chapter.

Similar to the results described in previous chapters, the collar also verified that the dogs slept most at night but spent most of their time inactive during the day. It also demonstrated that the higher patterns of activity happened in two specific points during the day, at 09:00 and at 18:00. Both are hours of high human movement around the facility due to management practices such as cleaning the kennels, feeding the animals and for the afternoon time, end of the shift for the day, when most staff are leaving. Furthermore, these times also correspond with the beginning and end of the students' classes in the University for the day, which increases traffic of people and vehicles around the kennels. All these factors could be contributing to arousing the animals and in response, producing longer bouts of high activity compared to other periods of the day.

Most importantly, the activity points acquired by the collars seemed to be constant in the way they measured activity, as no errant patterns were verified between days or between the same animals; that is, the collars were precise. The PetPace collar has been tested against a few other accelerometers that have been validated and are commonly used for scientific research in both humans and non-human subjects (Belda et al., 2018a; Ortmeyer et al., 2018).

For the most used brands, Actigraph and Actical, the collar achieved high levels of concordance between the data – 84% and 72%, respectively (Belda et al., 2018a) – meaning that it is a reliable tool for measuring activity.

The main difference between the PetPace and other monitoring devices is the fact that it provides real-time information if connected to a Wi-Fi network, however, there is the possibility of data loss if the collar is out of the range of the internet gateway for more than 12 hours. Since the kennels did not have internet connection available, we had to remove the collars from the dogs daily to download the data, which meant that for a period of time the collars were not acquiring any data from the dogs. Also, because we were not allowed on the kennels after work hours, our collars were out of range to transmit data for longer than instructed by the manufacturer, which generated some data loss (12% of total recorded behaviour). However, the loss did not have a significant effect on our results due to the extensive amount of time the dogs were monitored. For studies with smaller data collection periods, the loss of data could be a problem and proper planning should be taking into consideration to ensure data is being transmitted.

As reported in other studies, the collar is only able to detect HR and RR if the animal is resting or in low activity, not engaged in higher activity nor if there is too much barking/vocalizations happening (Belda et al., 2018a; Ortmeyer et al., 2018). Moreover, if the collar is loose, or the collar's case is displaced from the position on the neck recommended by the manufacturer, the sensor loses its ability to measure variation in HR and RR. This did happen with some of our subjects in several occasions. Nonetheless, this loss of data did not have an impact in our study as we were able to acquire a significant amount of data over time that represented the variation in such physiological measures in each dog.

Another interesting result was the similar patterns of HR and RR variation observed in paired dogs. In nature, several behaviours can be synchronized by animals: feeding, vigilance, reproduction, social cohesion, all with valid outcomes that benefit animals' groups (for review: Duranton & Gaunet, 2016). Some of these behaviours, such as reproduction, also cause synchronization of physiological measures, in this case oestrus cycles.

Dogs are a species with higher sensitivity to the changes in behaviour of other individuals, meaning they quickly pick up signals from co-specifics and adjust their own behaviour accordingly (Duranton & Gaunet, 2015). Moreover, recent research has found that dogs not only synchronize their behaviour (e.g. locomotion) with

humans but have similar long-term stress responses than their owners (Sundman et al., 2019). This could indicate that kennelled dogs may also synchronize behaviour and produce stress responses similar to their kennel mates.

5.5.1.1 Sleep parameters

The ability of the collar to measure behaviour had contradictory results. From the perspective of collecting rest points and generating an automatic report of sleep in their database the collar was efficient, especially if the data were transmitted live. Contrarily, as an indicator for sleep efficiency, adjustments are necessary. As to understand the architecture of dog sleep, the collar should be reporting sleep in duration, as it does with the other behavioural categories. Even though the collar does report different lying position in seconds, sometimes the animal can be inactive, but not necessarily sleeping.

Furthermore, it would be ideal to have a threshold that could account for the numbers of time that the dogs changed from sleep into wakefulness (sleeping bouts). As sleep fragmentation compromises both physiological and psychological health (Okun, 2011; Tobaldini et al., 2016), this feature could be an indication of increase or decrease in sleep quality when assessing the same individual over multiple nights. Additionally, this feature in a combination index with heart rate and/or respiratory rate could provide relevant information about the different sleep phases.

Previous studies that used accelerometer based technology to assess rest in dogs found different efficiencies in the methods. Clarke & Fraser (2016) reported an accuracy of 80-90% between the collar and observations when assessing rest based on head-down recumbency, which is a characteristic of sleep and sustained rest in dogs. However, inaccuracy sometimes happened if the dogs head was in an inclined angle, and if the dog was more restless during recumbency. Ladha & Hoffman (2018) had a similar approach to the problem evaluated by the previous study but added a rotation correction step designed to re-orient the sensor data as small movements were detected, hence the change in angle or sudden small movements did not cause an incorrect score, while providing information on head position during sleep.

The Pet Pace collar does not appear to have a problem with its rest threshold, since no resting points were scored with a value greater than zero. Nonetheless, a

point-based system to score sleep does not seem to be the most appropriate way of quantifying sleep or monitoring changing patterns. If the dogs is asleep but it moves, the collar will score the movement as some form of low activity, or if the dog is lying but awake, it will consider rest/sleep due to inactivity. Thus, producing inaccurate results.

5.5.2 Relationship between physiology, behaviour and sleep using the metrics acquired by the (PetPace) wearable technology collar

In our study, activity was highest during the day, in older dogs and correlated with higher levels of pulse and respiration. Similarly, standing and sitting had positive association with increased respiration rates, while time spent standing also increased the heart rate in the dogs. Sitting was also more expressed by older dogs. Sleep was most expressed during night and only associated with the rate of respiration, with no differences between younger and older dogs. Sleep duration was associated with the amount of standing and sitting the dogs would express during the day, with dogs sitting less, but standing more, in relation to longer hours of sleep at night.

The last behaviour measured eating/drinking was affected by period of the day, with dogs displaying more of this activity during daylight hours and associated with dogs' heart rate, which had elevated rates while feeding.

Domestic dogs have a clear diurnal pattern of activity with locomotion comprising from 60-75% of a 12-hour daytime period (Takeuchi & Harada, 2002; Zanghi, Kerr, DeRivera, Araujo, & Milgram, 2010; Zanghi, 2010). Activity can be affected by many factors in the environment, such as feeding times, but mostly dogs will express higher levels of activity between early morning and noon when compared to the second half of the day (Takeuchi & Harada, 2002; Zanghi et al., 2010; Zanghi et al., 2013a). As a diurnal species, sleep will be expressed mostly at night time; even so, as dogs are polyphasic sleepers, usually naps occur during the day (Iotchev et al., 2019; Zanghi, 2010).

These patterns of sleep/wake cycle found in previous studies corroborate with the measures taken from the (PetPace) collar. Yet, due to characteristics of the environment and an altered sleep architecture, discussed in earlier chapters, the dogs in our study did not sleep during the day. However, there was a higher level of

inactivity at this period, which indicates an attempt to mitigate the compromised sleep quality. Other studies found similar changes in activity patterns when sleep was disrupted in dogs

Ageing in dogs will also cause changes in the expression of activity, with senior dogs being observed to have less total activity during the day compared to younger adult dogs (Siwak et al., 2003; Takeuchi & Harada, 2002). In our study, age range (2-7 y.o.) was not sufficient to analyse differences between age groups, and probably the differences of sitting (less activity) and increase of activity with age could be an effect of the ratio of number of senior compared to younger dogs

Activity was also associated with increase in pulse and respiration and other studies with dogs have shown similar results. Ortmeyer et al. (2018) whose study also used the PetPace collar as a measure of behaviour/physiology, found higher rates in HR and RR with low and medium activity when compared with rest. They also found the same results for standing and sitting. Likewise, Maros, Dóka, & Miklósi (2008) found higher heart rates between activity and sedentary behaviour and also between lying and standing. It also found changes in pulse in relation to sitting and the presence or absence of a toy, which suggested that Heart Rate Variability could be an indicator of increased attention (Maros et al., 2008).

Other studies have explored the relationship between variation in pulse and an event that could have emotional valence, such as absence of owners or the presence of a positive stimulus (Katayama et al., 2016; Varga, Gergely, Galambos, & Kis, 2018; Zupan, Buskas, Altimiras, & Keeling, 2016). These findings concluded that heart rates does increase when dogs are separated from their owners (negative state) (Kuhne, Hößler, & Struwe, 2014; Maros et al., 2008; Varga et al., 2018), while respiration and heart rate decreased in the presence of a social reward (positive emotional state) (Katayama et al., 2016; Zupan et al., 2016).

As increases and decreases in heart rate and respiration rate are dependent on parasympathic nervous activation and emotions (see von Borell et al., 2007). The use of physiological variables to measure emotional states in response to activity or lack of activity in a specific environment could also be another way of measuring animal welfare.

Although we did not find any interference of sleep in HR data, and only a positive association with RR, other studies have investigated these matter and found effects in relation to stressful events (Bergamasco et al., 2010) and also between changes in HR after positive versus negative valence social interactions (Katayama et al., 2016; Zupan et al., 2016).

Lastly, we did find correlations between feeding and heart rate variability Dog food intake is mainly associated with time of feeding and quantity of the food (Zanghi, Kerr, de Rivera, Araujo, & Milgram, 2012; Zanghi et al., 2013b). Dogs fed once a day tend to change their locomotor and sleeping patterns in comparison to dogs fed twice a day (Zanghi et al., 2013b). Moreover, quantity and quality of food can lead to anticipatory feeding behaviour and obesity, which can have negative impacts in physiology, such as elevated heart rate (Degeling, Rock, & Teows, 2011; McMillan, 2013). Emotional eating has also being investigated in animals, although not extensively, and findings of overfeeding are related to stress, anxiety, boredom and lack of mental stimulation (as reviewed by McMillan, 2013). These are also common indicators of poor animal welfare in most animals, including dogs (Polgár et al., 2019; Yeates, 2016).

5.5.3 Evaluating the efficiency of the (PetPace) wearable technology collar against behavioural observations

The results produced by the collar showed the same distribution of activity and sleep as found in previous chapters. However, the amount of time quantified by the collar versus the amount of time reported by the observations did not find the same results when allocated in the three broad categories used for the analysis. Only inactivity was scored the same by the different methods.

Most likely, this difference may be occurring due to the different metrics applied between the methodologies, as well as the categorization the collar has, which is different from the ones scored by the observers during the videos.

For the nocturnal behaviours, as the data were being scored continuously by the observer and the goal was to identify patterns of sleep, only three categories were used during the video analysis: Active, inactive and sleeping, while the collar continue to score seven categories due to its setup. Nonetheless, we found similarities between

the results of each method. During the day, the observations were conducted with the aid of an ethogram and used more behavioural classes than the collar. As well, the amount of time the behaviour was being recorded manually was significant less than the recordings made by the collar.

To improve accuracy between the methods for the diurnal observations, probably a sample point by sample point analysis of behaviour should be used instead of percentages by day. Meaning each observation made manually would have to be matched by the exact time from the collar. Although, this would be a very time-consuming task, as opposed to matching only means for the same days, (as it was done for the analysis in this chapter).

When using the values of points-based system of the collar converted into a ratio, we were able to find more appropriate results than using the ones from the previous analysis, especially when looking at the mean variation of sleep and activity in individual dogs. As activity and rest were only scored as points, not as duration of behaviour (e.g. sleep duration or locomotion duration) the calculation of the ratio appears to have provided a more comparable result than an activity threshold to conventional observational methods. When evaluating animal welfare, the quantification of total time of a certain behaviour provides a more reliable information than an activity score, as the output provided by PetPace. Still, if the scoring can be monitored over time individually, the variation in activity levels could indicate behavioural problems (i.e. increase in one specific behaviour score) and this would be a good alternative to watching several hours of video.

Overall, when analysing the inaccuracy of the collar compared to the behavioural observations, none of the compared categories had more than 17% of difference in the results, which in a long-term project would not cause a problem in the analysis. However, for smaller sampling sizes the margin of error could be problematic and cause a bias on the results. Furthermore, for some specific behaviours such as sleep, the collar still needs further refinement to be used as an alternative to behavioural observations.. .

5.6 Conclusion

Our results demonstrated that using an accelerometer-based technology in combination with health-related measures could prove to be a useful tool to assess animal welfare remotely. However, the acquisition of more detailed information such as measuring sleep quantity and fragmentation needs more refinement. Nonetheless, the sleep score index generated automatically by the collar's software could provide a preliminary assessment of sleep and could be useful to monitor broad variations in sleeping patterns. Additionally, ours results illustrated that the merged data was effective in establishing relationships between behaviour and physiological measures. Considering the collar is a non-invasive method, the ability to explore such relationships effectively and in a simple way, provides an alternative to study welfare in dogs.

Further research is still necessary to understand the effects of the changes in physiology in relation to the environment, sleep, and stress responses in dogs. Nevertheless, this initial investigation brings new and relevant information to the welfare (especially the relevance of emotional states) of kennelled dogs.

Chapter 6. Automated observations of dogs' sleep patterns using Convolutional Neural Networks and computer vision

6.1 Introduction

The discipline of animal welfare relies on, amongst other things, the quantification of animal behaviour, because an individual´s behaviour is a direct response of its attempts to adapt to its environment (Broom, 1991; Hill & Broom, 2009). Moreover, as a non-invasive measure, it holds an advantage over other measures such as physiological parameters, which can affect the variable to be measured (Hill & Broom, 2009; Mason & Mendl, 1993). Common animal welfare measurements of behaviour include: the assessment of time budgets; changes in natural behavioural patterns (e.g. increased inactivity); expression of abnormal behaviours; inappropriate social and agonistic responses# (Broom, 1991; Mason & Latham, 2004; Shepherdson, 2012).

Although a simple method, behavioural assessments are not without their limitations. First, observing behaviour demands time from a human observer to quantify the behaviour. Second, to answer specific questions related to welfare, at least a basic knowledge of the species´ behaviour is necessary. Third, results are highly dependable on reliability of scoring the same behaviours consistently over time, which demands training of human observers to ensure inter-observer reliability. Lastly, not all animal holding institutions allow researchers to observe animals outside working/daytime hours, which causes loss of important information over time; for example, zoo studies are biased towards daylight hours (Fonio, Golani, & Benjamini, 2012; Levitis, Lidicker, & Freund, 2009; Mason & Mendl, 1993).

With the advances of technology, it is possible to try to mitigate some of the aforementioned problems; for example, video monitoring of animals in different environments used as an alternative to direct observations but images still need to be quantified by a human observer (Anderson & Perona, 2014; Egnor & Branson, 2016). Additionally, software has been developed to help scoring of behaviour from the videos

to expedite the processing of images (e.g. BORIS, Friard & Gamba, 2016). Despite this, human-observed measurements of animal behaviour, even when computer assisted, remain slow, labour intensive and a prone to errors (Anderson & Perona, 2014; Button et al., 2013; Fonio et al., 2012). Such tedious and long processes reduce the number of experiments that can be conducted, reduce the opportunity to work with larger sample sizes and can limit statistical power (Anderson & Perona, 2014; Button et al., 2013).

With recent advances in computational vision and deep learning, automated tracking and behaviour analysing systems have the potential to change the way behavioural variables are measured. The use of automated video analysis can increase scoring accuracy, rigor of experimental design, increase the number and nature of variables being measured, allows the generation of larger data sets, and enables larger samples sizes, which increases statistical power (Barnard et al., 2016; Pons, Jaen, & Catala, 2017; Valletta, Torney, Kings, Thornton, & Madden, 2017). These are the aims of the emergent field *of computational behaviour analysis* (Egnor & Branson, 2016), also referred to as computational ethology (Anderson & Perona, 2014).

Automated video systems to record animal behaviour already exist for wild animals (Gomez Villa, Salazar, & Vargas, 2017), farm animals (Rushen et al., 2012), laboratory rodents (Van De Weerd et al., 2001), insects (Noldus, Spink, & Tegelenbosch, 2002), fish and even as well-developed commercial systems such as Ethovision (Noldus, Spink, & Tegelenbosch, 2001). Furthermore, in the field of animal welfare such systems have being used to monitor pregnant cows before calving (Cangar et al., 2008), aggression in pigs (Oczak et al., 2013), and the activity of broiler chickens with different gait scores (Marian Stamp Dawkins, Cain, & Roberts, 2012).

In the context of dogs, most autonomous measurements rely on wearable technology for pets (Jukan, Masip-Bruin, & Amla, 2017b), such as the PetPace collar described in Chapter 5. While these devices can measure activity and sleeping patterns, scientific validation is often lacking (Belda et al., 2018a; Weiss et al., 2013), and it is not always appropriate to use this technology in clinical or scientific settings. Moreover, although some of these sensor-based activity trackers have achieved good accuracy (Belda, Enomoto, Case, & Lascelles, 2018b; Ladha & Hoffman, 2018b;

Olsen et al., 2016), they are limited to a small number of basic behaviours (e.g., resting) and postures (e.g., lying down, sitting, etc).

Despite being a well-studied species, only a few studies address the use of automatic video-based analysis of dog behaviour (Amir, Zamansky, & van der Linden, 2017; Baba, Pescaru, Gui, & Jian, 2016; Barnard et al., 2016; Mealin, Domínguez, & Roberts, 2016). The studies were successful in automatic tracking of the individuals as well as detecting dogs' body parts using machine learning classifiers. However, the experiment used videos taken from 3D Kinect cameras (Microsoft) or using street surveillance systems whose installation is not trivial, and the devices are expensive.

In this chapter, we present a system developed in partnership with the University of Haifa in Israel, which combines convolutional neural networks (CNN) with classical data processing methods to automatically detect and quantify dogs' sleeping patterns. A CNN is a deep learning algorithm, which in this case is trained to classify behaviours directly from images. These networks learn from image data how to use patterns to identify objects, faces and scenes (Karpathy et al., 2014). Differently from previous studies using automatic video analysis, which required specialized equipment, this system was designed to work on video footage obtained from low quality, cheap and easily available cameras. It also has a user-friendly interface that produces a summary output of the variables measured, which removes the need for any advance knowledge from the user to be able use the system.

6.2 Aim

To develop and validate an automatic video-based monitoring system to quantify dogs' sleeping patterns and verify the accuracy of such system against human observations.

6.3 Methods

6.3.1 Video acquisition of dogs

The footage used to train the Network and develop the system was the one captured during the eight months in which the study with sleep patterns of laboratory

dogs was being conducted. Further description of the subjects and kennel settings can be found in Chapter 3.

The videos were recorded in a domestic CCTV system (Swann SWDVK-845504) with night vision capability. The cameras were able to capture videos in two modes: full-colour mode, when the sun or a lamp illuminates the space, and grey-scale mode, during the night (dark) or with very low lights levels, which triggers the infrared light and automatically switches the camera to night vision. Despite the HD resolution (1280x720) of the cameras, the video footage is technically considered as of low quality.

In total, 13,668 videos were captured by the cameras, totalling over 4000 hours of footage. All the footage was sent to the '*Tech 4 animals*' lab at the University of Haifa Israel, for development of the system.

6.3.2 Blyzer^{DS} System Overview

The system Blyzer^{DS} (Behavior Analyzer – Dog Sleep; Figure 40) is an extension of a previously developed system for automatic tracking of dog movement (Amir et al., 2017), which was tailored specifically for the needs of this project.

The system takes as input digital video footage and produces a summary of sleep parameters: currently it supports calculating the amount of sleep of each dog in the footage, and number of sleeping bouts. The video is sent to the system server and processed frame by frame. The frames are analysed by the Neural Network, which has two main tasks: marking a dog (or dogs) position and classifying the dog (or dogs) state as awake or asleep (Figure 40).

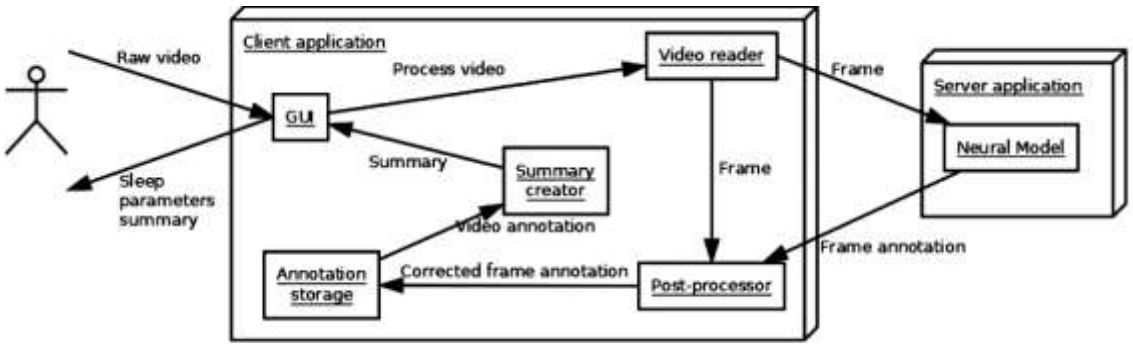

Figure 39. Overview of the system's client/server architecture for the automated analysis of dog sleeping behaviour using Convolional Neural Networks.

6.3.3 Neural Network analysis

The input to the system is raw digital video, and its output is a summary of the sleep parameters for that video. The analysis is performed in two stages:

Stage 1. The system application sends the raw digital video to the server frame by frame. The server application uses a previously trained neural network to detect a dog (or a number of dogs) in a frame, and outputs a bounding box around it (Figure 41). The frames with the bounding box are returned to the system application.

Figure 40. Detection of dogs by the Blyzer^{DS} system using a Neural Network. Different colours in the bounding boxes show the system correctly scoring two individuals.

Stage 2. The client then proceeds to detect movement in a series of frames (Figure 42). If no movement is detected for a certain amount of time (currently the parameter is set for 0.5 seconds), the dog is classified as asleep.

The movement detection steps are:

1. Conversion of the bounding box content to black and white image.
2. Blurring of the image.
3. Calculate delta between consecutive frames.
4. Binarize computed delta using a threshold.
5. Dilate the binarized image to fill in holes.
6. Detect contours and compute their area.

Figure 41. Motion detection steps by the Blyzer^{DS} system (a computer system to automatically score behaviour of animals). First row: two consecutive frames turn black and white. Second row: Blurring the images. Third row: Dilation of frames and detection of contours

After these steps, the system scores the dog as being asleep or awake and returns a summary of sleep parameters for that video. Further and more detailed information of the system can be found in Zamansky et al (2019), in the appendix section (Appendix 2).

6.3.4 Training data set and system evaluation

The system was trained using 8000 frames extracted from the data set. The developers manually revised the output results to ensure each frame contained the two attributes: a bounding box surrounding each identifiable dog and the state of the dog as being awake or asleep. Any frames with unclear image of dogs, or with no dogs were discarded from the analysis.

The accuracy of the system was evaluated using 10 videos of 600 seconds each. The video set included videos with 0-2 dogs, day/night time and different dogs and kennels. The videos were processed by the system and a testing set of 6,000 frames annotated with the system's predictions were manually checked for correctness by the developers.

6.3.5 Verifying the accuracy of the system against the behavioural observations

Due to the nature of this project, the system took a long time to be ready to be tested against the behavioural observations. As the system is still being refined for accuracy since sometimes, it classifies movement incorrectly when two dogs are in close proximity to each other, or laying down together, we decided to use only the data recorded for Atila (the dog housed alone). In total, 15 nights were submitted to be evaluated by the system. The system calculated the duration inactivity per night and generated the number of bouts of sleep per night recorded.

6.3.6 Statistical Analysis

The summary results from the system and the behavioural recordings were tested for normality using Anderson-Darling tests. All statistical tests were considered significant at $p<0.05$. Results are presented as either total measured time or score totals. The system efficiency was tested against the human recorded observations of

the same observation sessions using Paired t-tests (Dytham, 2011). Statistical analyses were carried using Prism 8 (GraphPad Software, 2018).

6.4 Results

During the evaluation of the system using the training data set, the system classified correctly 5340 frames out of the 6000 tested, scoring 89% accuracy.

From the 15 days submitted, 3 days had to be excluded from the final analysis since bad weather conditions caused the cameras to move; as well the lights to switch on and off leading to inaccurate analysis. An additional day was excluded due to the loss of 3 hours of footage, which prevented comparisons between the methods.

The system scored an average of sleep of 10.9 ± 2.2 hours, against 9.7 ± 1.6 hours recorded manually by human observers. Moreover, the system found an average of 15 ± 5 bouts per night, while the human observations returned 16 ± 3.5 bouts per night. Differences between the methods ranged from 0.13% to 2.68%, with a mean difference of 0.88%. Results are summarized in the table below:

Table 17. Summary of sleep metrics recorded by the Blazer^{DS}system and compared with the recordings from manual (human) observations for the dog Atila for 10 nights.

Sleep System	Sleep Manual	% difference	Bouts System	Bouts Manual	% difference
12:05:56	09:31:33	1.15	14	15	2.94
12:52:49	10:41:49	0.71	7	15	23.53
11:42:26	09:59:51	0.39	19	17	5.88
11:15:11	10:04:42	0.06	22	20	5.88
12:37:12	09:41:49	1.42	12	15	8.82
05:26:04	04:58:31	0.13	10	8	5.88
11:43:39	09:58:31	0.43	15	15	0.00
10:48:40	11:05:58	1.38	23	19	11.76
11:56:16	11:15:38	0.59	12	21	26.47
12:20:21	10:10:57	0.74	13	16	8.82
08:05:47	10:05:20	2.68	18	18	0.00

Using the absolute values (duration in seconds) there was a significant difference between the computer system and the manual recordings (W = 40, p = 0.04; Figure 43). However, when the recordings were converted to percentages of time observed, no statistical difference was found between the methods (p>0.05; Figure 44).

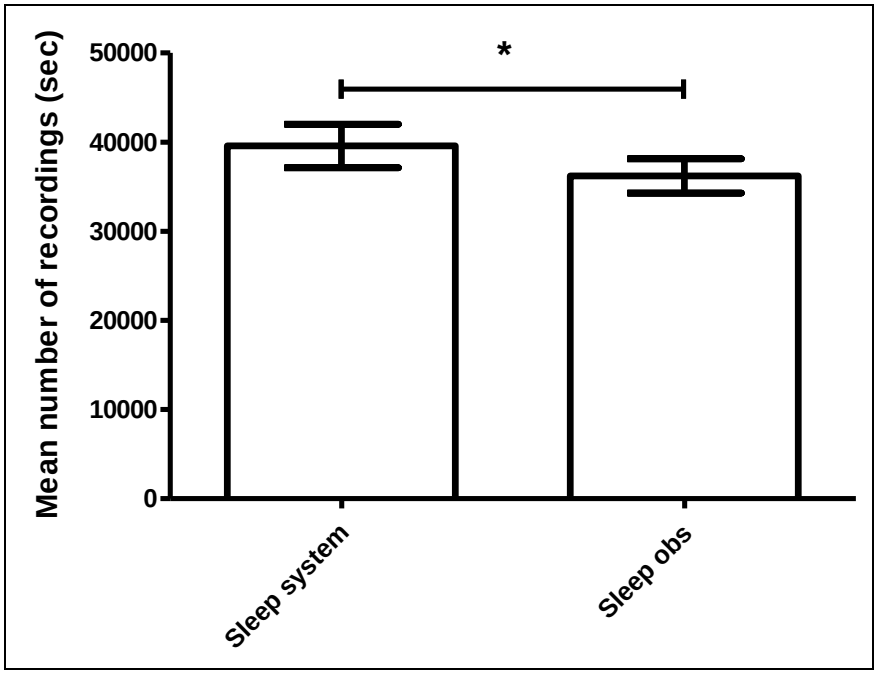

Figure 42. Comparison of mean duration of sleep between the manual (human) observations and the Blazer^{sd} System (computer observations). Line above columns with star indicate significant differences at p<0.05. Error bar show ± standard error of the mean.

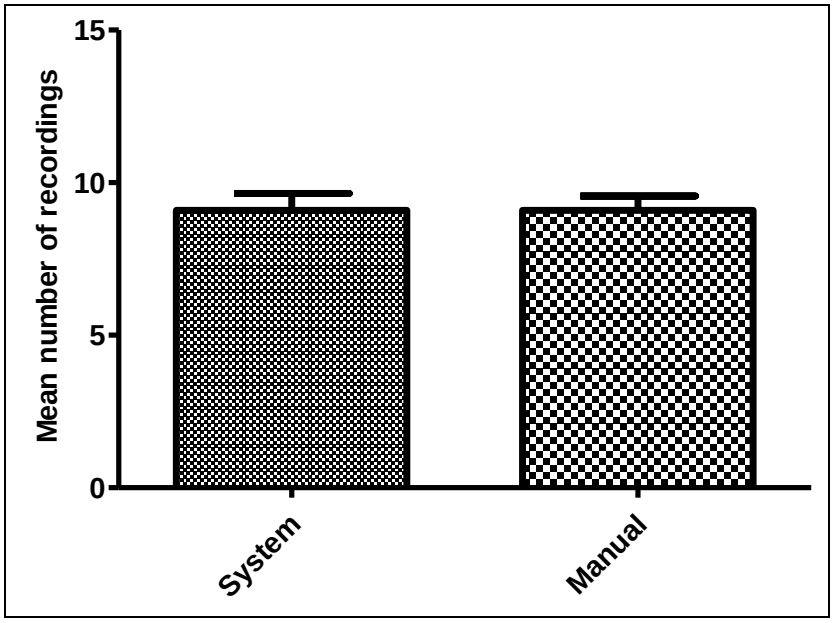

Figure 43. Comparison between of mean time observed (percentage) of sleep behaviour scored by the Blazer^{SD} System (computer observations) and manual recordings (human observations). Error bar show ± standard error of the mean.

6.5 Discussion

Automating the monitoring of behaviours offers a practical solution for mitigating common problems associated with the measure of animal behaviour and animal welfare (Anderson & Perona, 2014; Button et al., 2013; Fonio et al., 2012). By using a system based on the recent advances in artificial intelligence (A.I.), it is possible to optimize data collection, increasing accuracy, objectivity and experimental throughput (Barnard et al., 2016; Egnor & Branson, 2016; Valletta et al., 2017). Furthermore, these systems could become a valuable instrument for monitoring behaviours that are hard to assess such as resting (Pons et al., 2017; Valletta et al., 2017). As A.I. continues to learn while it is being used, it is possible that it could pick up patterns of small changes of activity (e.g. small twitches) or find patterns in changes in body postures that could be associated with the shifts of different sleep phases (i.e., from non-REM to REM).

Quantifying sleep and rest behaviour through video monitoring has been proven to be an effective non-invasive technique, with some studies scoring over 90% of confidence between observations and EEG (e.g. Balzamo, Van Beers, & Lagarde, 1998; McShane et al., 2012). Although the video monitoring was based on human observations, it stills shows that it is possible to measure sleep using a video-based methodology. Hence, the automatization of such process would be beneficial to the field of animal welfare.

In this project, our main goal was to seek a multidisciplinary approach in order to evaluate sleep as a measure for animal welfare, but ultimately, the ideal was to find alternatives to the invasive techniques used to acquire such information. Video monitoring is an invaluable tool for such studies since cameras provide a spatial and temporal metric that in principle can assess most aspects of animals' behaviour without interfering with the individuals (Anderson & Perona, 2014; Fontaine et al., 2008). However, the downside of using this methodology in our research was the extensive hours of video monitoring due to the amount of data recorded, to which, the Blazer^{DS} System, based on convolutional neural network analysis, provided an efficient and accurate solution. The system is still undergoing development to become more accurate and precise in the measurement of sleep parameters. Mostly, the

problems associated with processing the images are related to image quality and due the presence of ‘noise’ in the footage. For example, as the cameras are exposed to the weather, in windy days they move significantly, blurring the images, which leads to inaccurate measuring. The poor lighting conditions at the kennels also made it difficult to identify the dogs if they were in darker corners. These conditions can be mitigated by adjusting the algorithm to compensate for movement and light, although this will require several months to be achieved.

Nonetheless, in the days the system was able to record more sleep than the manual observations, brings to light the problems associated with manually going through thousands of hours of recordings. Human observers usually take up to three times the time of a video to be able to score it, which will cause tiredness leading to inconsistency of measurements over time (Anderson & Perona, 2014; Button et al., 2013). Hence, the use of automatic systems has the potential to change the way scientists conduct research in this field today.

The use of CNN based systems such as the Blazer^{DS} system with further improvements and new training data sets can be modify to record more categories of behaviour and the behaviour of other species. This could lead to a system that can be broadly used by people working with animal in different environments and that can have a positive impact on the welfare of domestic, farm and zoo animals.

6.6 Conclusion

The use of CNN based systems such as the Blazer^{DS} system have the potential to change the way we conduct research in animal behaviour and welfare. Even in its early stages, such systems show a good level of precision and accuracy when evaluating the sleep behaviour of dogs. The development of autonomous system for behaviour analysis helps mitigate common problems associated with processing manually large video data sets, an extremely time consuming, tedious and error-prone task.

Chapter 7. General Discussion

The aims of this thesis were to explore the use of sleep as a measure of animal welfare using the domestic dog as a model mammal species. A further aim was to investigate the use of technology to automatically record sleep; thereby making the assessment of sleep in animals a practical reality.

7.1 New Contributions to Science

This research project has presented a general overview of dogs' sleep welfare, considering new methodological and experimental approaches and envisioning the use of refined technologies to access sleep quantity and quality. Described in the subsections below are the main findings and contributions of each one the experimental chapters produced using the results of this research.

7.1.1 Chapter 2 - Basic characteristics of sleep-related behaviours of kennelled laboratory dogs

Δ Dogs in our study had altered sleep architecture:

One of the main findings of this chapter was the evidence that the dogs in the kennels had sleep loss during the day and fewer sleep cycles at night in comparison to other studies.

Δ Dogs modified sleep to try and adapt to the environment:

The dogs in our study did not show any signs of sleep compensation (e.g. increased sleeping times) after nights when the amount of sleep was significantly reduced. Instead, a large amount of inactivity was recorded during most part of daytime.

Δ Individual variance is an important component of sleep welfare:

Sleep was found to be very plastic between individuals, with some dogs having more compromised sleeping cycles that others (i.e. male and younger dogs).

7.1. 2 Chapter 3 - How sleep fragmentation affects overall behaviour in laboratory kennelled dogs

Δ Sleep fragmentation has a negative impact on behaviour:

Sleep fragmentation as a result of sleep deprivation in this study was related to the expression of other behaviours during the daylight period.

Δ The behaviours impacted by sleep loss can affect physiological and psychological variables in the dogs:

Different behaviours such as eating, locomotion, alertness, inactivity and play were all negatively impacted by the compromised sleeping patterns of the dogs.

7.1.3 Chapter 4 - Do environmental conditions affect sleep in kenneled dogs?

Δ The kennel environment trigger stress responses in the dogs, which are exacerbated by sleep deprivation:

During the day, multiple factors in the kennels such as light, noise and temperature had a direct impact on the dogs' cortisol levels; this associated with the lack of sleep appears to compromise dogs' physical and psychological health in the kennels.

Δ Noise in the kennels was the variable that most affected the dogs:

Loud days directly affected dogs' rest and increased cortisol levels, which exacerbated stress responses and consequently more disturbed sleep.

7.1.5 Using wearable technology to measure sleep behaviour and general physiology of laboratory dogs

Δ Measuring behaviour and physiological variables without invasive techniques:

The outcome of using a wearable sensor on the dogs was the ability to extract such data remotely without any negative consequences for the animals.

Δ **Using wearable technology to measure sleep:**

Data were found to be similar between the human-observed measures and the scoring generated by the (PetPace) collar, which indicates the system is accurate enough to be use as an alternative to video-based observations.

Δ **Understanding the interpolation between physiological measures acquired by the collar and sleep:**

Our results did not provide enough consistent results to associate physiological measures with sleep, however, the ability to gather such information can be relevant while monitoring animals over longer periods.

7.1.6 Automated observations of Dogs' Sleep Patterns using Convolutional Neural Networks and Computer Vision

Δ **The development of an automated system to measure sleep in dogs:**

The system developed passed its proof of concept stage that computer vision based on convolutional neural networks can accurately record the sleep behaviour of dogs and thereby in the future could automate the recording of this behaviour.

7.2 Experimental limitations

The major limitation in this project was the sample size. Although the long-term monitoring of the dogs provided an enormous quantity of data it is still based on the same individuals in a specific setting, which is a limitation when comparing the results with other studies, or when verifying the applicability of the methods currently used for different dog populations. Further limitations in the research based on the different methodologies used are:

Δ Inability to score manually or with automated technology the different phases of sleep. For example, the duration of REM sleep.

Δ Lack of representation of different age groups (i.e. a wider age range).

- Δ The ability to manipulate experimental conditions. One of the initial ideas of the project was to have different settings (e.g. barren kennels vs Enriched Kennels) to see how sleep changes with the increase of welfare. This was not possible due to restrictions imposed by kennels staff.

- Δ Data loss due to equipment fault for both the collars and the cameras.

- Δ Limitations in the statistical analysis caused by problems of singularity in the linear models when using kennels as a variable to mitigate the possibility of pseudoreplication.

- Δ Interference on experiments due to the easy access of students to the kennels facilities.

- Δ Dogs' ability to remove collars from each other and damaging the equipment.

- Δ Collar activity output being scored through a point-based system, which limits the interpretation of different types of activity (e.g. duration of locomotion in opposition to type of activity).

- Δ Training the Blazer^{DS} system network demands an extensive amount of time and results are highly affected by changes in light conditions and camera movements.

- Δ When using multiple techniques evaluating the same goal, methods metrics and outputs must be considered in the experimental design, in order to make the analysis of results more efficient and precise

7.3 Future directions for investigating sleep welfare

The results presented in the experimental chapters have shown the need to include sleep measures in animal welfare assessments, due to the importance of this behaviour for the dogs' quality of life, and which significance can also be extrapolated to other species. Here are some suggestions for future research based on this study:

- Δ Investigate the effects of sleep disruption on species with different durations of sleep

- Δ Investigate the effects of sleep disruption on species with different sleep architecture (e.g., monophasic versus polyphasic)
- Δ Manipulation of kennelled dogs' environment to determine how sleep is affected (e.g. implementation of walks vs dogs that do not walk)

- Δ Quantification of REM versus non-REM sleep in the aforementioned studies

- Δ Refinement of algorithms of wearable technology (e.g., PetPace collar) to score sleep and different types of active behaviour such as locomotion in duration in seconds

- Δ Development of an index based on combined measures of physiology and behaviour acquired by the pet pace collar to score sleep quality

- Δ Autonomous monitoring of different environmental conditions such as noise or light simultaneously with sleep behaviour

7. 4 Final Conclusion

As the demand for simple, improved, and financially accessible methods to assess animal welfare continuous to increase, the automated monitoring of sleep behaviour using computer vision based on convolutional network analysis could shift the direction in animal welfare research by allowing automated real-time monitoring of animal welfare in a broad range of different animal species under human care.

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Appendices

Apendix 1 - Example of GLMM model in R in that included Kennel as a random factor and that returned a singularity warning

```
> dados <- read.csv("Canil noite.csv", h = T)
> names(dados)
 [1] "date"              "Subject"           "Kennel"            "Sex"
"Age"
 [6] "Active"            "Inactive"          "Sleeping_duration"  "Sleep
ing_right"     "Sleeping_left"
[11] "Sleeping_sternally" "Sleeping_back"     "Sleeping_Bouts"     "Laten
cy"            "Duration_Awake"
[16] "Cortisol"          "dB"
> scaled=scale(dados[,6:17],center = T, scale = T)
> summary(scaled)
     Active            Inactive        Sleeping_duration Sleeping_right
Sleeping_left      Sleeping_sternally
 Min.   :-1.55550   Min.   :-1.39808   Min.   :-1.7066   Min.   :-1.36001
Min.   :-1.21699   Min.   :-0.2761
 1st Qu.:-0.57532   1st Qu.:-0.85441   1st Qu.:-0.8421   1st Qu.:-0.94494
1st Qu.:-1.08599   1st Qu.:-0.2761
 Median :-0.08424   Median : 0.03463   Median : 0.4183   Median : 0.05671
Median :-0.02185   Median :-0.2761
 Mean   : 0.00000   Mean   : 0.00000   Mean   : 0.0000   Mean   : 0.00000
Mean   : 0.00000   Mean   : 0.0000
 3rd Qu.: 0.44877   3rd Qu.: 0.64521   3rd Qu.: 0.7976   3rd Qu.: 0.83104
3rd Qu.: 0.70362   3rd Qu.:-0.2761
 Max.   : 7.08382   Max.   : 2.54439   Max.   : 1.2541   Max.   : 1.91567
Max.   : 3.19451   Max.   : 8.6603
                                                        NA's   :1
NA's   :1          NA's   :1
 Sleeping_back     Sleeping_Bouts       Latency         Duration_Awake
Cortisol               dB
 Min.   :-0.1529   Min.   :-1.5450   Min.   :-0.47512   Min.   :-0.8577
Min.   :-0.84950   Min.   :-2.84647
 1st Qu.:-0.1529   1st Qu.:-0.8322   1st Qu.:-0.44618   1st Qu.:-0.8577
1st Qu.:-0.46774   1st Qu.:-0.56575
 Median :-0.1529   Median : 0.1657   Median :-0.30739   Median :-0.4815
Median :-0.26102   Median : 0.03016
 Mean   : 0.0000   Mean   : 0.0000   Mean   : 0.00000   Mean   : 0.0000
Mean   : 0.00000   Mean   : 0.00000
 3rd Qu.:-0.1529   3rd Qu.: 0.7360   3rd Qu.: 0.05396   3rd Qu.: 0.9325
3rd Qu.: 0.09073   3rd Qu.: 0.84684
 Max.   : 8.7624   Max.   : 1.7339   Max.   : 6.70632   Max.   : 2.7278
Max.   : 6.60236   Max.   : 1.36307
 NA's   :1
NA's   :15         NA's   :14
> mydata=data.frame(dados,scaled)
mydata=na.omit(mydata)
> t1 <- lmer(Active ~ Sleeping_Bouts+Latency.1+Duration_Awake.1+Cortisol+d
B+Sleeping_right.1+Sleeping_back.1+Sleeping_sternally.1+Sleeping_left.1+(1
|date)+(1|Kennel), data = mydata)
boundary (singular) fit: see ?isSingular
> summary(t1)
Linear mixed model fit by REML ['lmerMod']
Formula: Active ~ Sleeping_Bouts + Latency.1 + Duration_Awake.1 + Cortisol
+
    dB + Sleeping_right.1 + Sleeping_back.1 + Sleeping_sternally.1 +
Sleeping_left.1 + (1 | date) + (1 | Kennel)
   Data: mydata

REML criterion at convergence: 2341.6
```

```
Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.2834 -0.6534 -0.1099  0.5526  4.2555

Random effects:
 Groups   Name        Variance  Std.Dev.
 date     (Intercept) 6.505e-02    0.2551
 Kennel   (Intercept) 2.413e+05  491.1898
 Residual             1.061e+07 3256.5332
Number of obs: 130, groups:  date, 34; Kennel, 7

Fixed effects:
                    Estimate Std. Error t value
(Intercept)          4671.090   3219.837   1.451
Sleeping_Bouts        288.513     73.554   3.922
Latency.1               3.648    277.776   0.013
Duration_Awake.1      588.018    343.227   1.713
Cortisol               29.860     45.241   0.660
dB                    -28.418     48.851  -0.582
Sleeping_right.1     -996.878    411.353  -2.423
Sleeping_back.1      1211.492    280.541   4.318
Sleeping_sternally.1 -193.705    397.109  -0.488
Sleeping_left.1      -530.459    395.027  -1.343

Correlation of Fixed Effects:
            (Intr) Slpn_B Ltnc.1 Dr_A.1 Cortsl dB     Slpng_r.1 Slpng_b.1
Slpng_s.1
Sleepng_Bts -0.222
Latency.1   -0.035  0.023
Durtn_Awk.1 -0.074 -0.168 -0.035
Cortisol    -0.098 -0.093  0.053  0.257
dB          -0.960 -0.020  0.019  0.094  0.029
Slpng_rgh.1  0.196 -0.626  0.126 -0.024  0.085 -0.054
Slpng_bck.1 -0.134 -0.195  0.054 -0.118  0.045  0.182  0.183
Slpng_str.1 -0.234 -0.116 -0.120  0.047  0.128  0.264 -0.015     0.087
Slpng_lft.1  0.162 -0.539  0.057 -0.225 -0.016 -0.029  0.256     0.160
0.104
convergence code: 0
boundary (singular) fit: see ?isSingular
anova(t1)
Analysis of Variance Table
                    Df    Sum Sq   Mean Sq F value
Sleeping_Bouts       1 223168484 223168484 21.0437
Latency.1            1    494651    494651  0.0466
Duration_Awake.1     1  46683955  46683955  4.4021
Cortisol             1   6412438   6412438  0.6047
dB                   1  26644933  26644933  2.5125
Sleeping_right.1     1  89833560  89833560  8.4709
Sleeping_back.1      1 227246929 227246929 21.4283
Sleeping_sternally.1 1   1295463   1295463  0.1222
Sleeping_left.1      1  19123193  19123193  1.8032
```

Appendix 2

Analysis of Dogs' Sleep Patterns Using Convolutional Neural Networks

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Abstract. Video-based analysis is one of the most important tools of animal behavior and animal welfare scientists. While automatic analysis systems exist for many species, this problem has not yet been adequately addressed for one of the most studied species in animal science—dogs. In this paper we describe a system developed for analyzing sleeping patterns of kenneled dogs, which may serve as indicator of their welfare. The system combines convolutional neural networks with classical data processing methods, and works with very low quality video from cameras installed in dogs shelters.

Keywords: Convolutional neural networks · Animal science · Animal welfare · Computer vision

1 Introduction

Video-based analysis is one of the most important tools of animal behavior and animal welfare scientists. For instance, it is very useful for measuring *time budget* of animals, a common ethological and welfare parameter, indicating the amount or proportion of time that animals spend in different behaviors [1]. In this case the data to be analyzed may amount of hundreds of hours of data, and is a tedious and error-prone task. Naturally, automatic video analysis has the potential to revolutionize the work of animal scientists in terms of precision, nature and number of behavioral variables that can be measured, and volumes of video data that can be processed. Automatic video-based systems already exist for different species: wild animals [2], pigs [3,4], poultry [5], insects [6], and many more. Moreover, well-developed commercial systems for rodent tracking such as Ethovision [7,8] are widely used in behavioral research.

Dogs are a widely studied species in animal science. While video analysis is widely applied in the context of dogs (see, e.g. [9,10]), very few works address

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automatic video-based analysis of dog behavior [11–13]. All of these works use video from 3D Kinect camera, the installation and use of which is not trivial and also quite expensive.

Our approach takes a different strategy, using the simplest web or security cameras footage, and paying a "computational" price instead for the system's learning. It is a part of our ongoing multi-disciplinary project for automatic analysis of dog behavior, based on video footage obtained from simple cameras (an overview of the project can be found in [14]; preliminary ideas were presented in [15]). In this paper we present a system developed for supporting an ongoing research project in animal science, investigating sleeping patterns of kennelled dogs as indicators of their welfare. Our system was developed for automatically quantifying dogs' sleeping patterns. It combines convolutional neural networks with classical data processing methods; it works with very low quality video data, and supports detecting multiple dogs in a frame. In what follows we describe in further details the research problem and the developed solution.

2 Related Work

Automatic tracking and behavior analyzing systems are used for wild animals [2], pigs [3,4], poultry [5], insects [6], and many more. Well-developed systems for rodent behavior recognition such as Ethovision [7,8] are widely used in behavioral research. In the context of dogs, automatic quantification of animal activities have mostly been explored in relation to pet wearables. These include a plethora of commercially available canine activity trackers (such as FitBark[1], Whistle[2] or PetPace[3]). While such devices can measure activity and sleep patterns, none of them has yet been scientifically validated, and thus are not always appropriate to be used in clinical and scientific settings. Wearables have been investigated in the context of predicting the success of future guide dogs ([13,16]), impacting the bonding between dog and owner [17,18], and supporting the relationship between guide dog centers and puppy raisers ([19]). van der Linden et al. [20] provide a comprehensive overview of commercially available dog trackers, discussing also their privacy implications. yet ripe to be used for scientific research or clinical settings. Fair accuracy was achieved for several self-developed sensor-based activity trackers [21–23], which are limited to a small number of basic positions and postures.

Barnard et al. addressed a similar problem of automatic behavioral analysis of kennelled dogs using 3D video monitoring [11]. Dog body part detection was done using standard Structural Support Vector Machine classifiers, and automatic tracking of the dog was also implemented. However, as discussed in the introduction, this approach requires expensive equipment and non-trivial installation of 3D cameras (such as Microsoft Kinect). Our approach, on the other

[1] See: https://www.fitbark.com/.
[2] See: https://www.whistle.com/.
[3] See: https://petpace.com/.

hand, can use video footage of very low quality, obtained from simple, cheap and easily available cameras.

3 Problem Definition

The above mentioned animal science study[4] is a collaboration between the University of Salford and the Animal Science Center of Universidade Federal de Ouro Preto in Brazil. It focuses on analyzing sleep patterns of breeding stock kenneled dogs as welfare indicators. The dogs, bred and maintained by the Animal Science Center in Brazil, were captured for eight consecutive months using simple security cameras installed in their kennels (using night vision at night). The collected video data is of size 2.1 TB and contains 13,668 videos, comprising over 4,000 hours of footage. Each of the kennel rooms house either one or two dogs. The cameras are able to capture videos in two modes: full-color mode, where the space is illuminated by the sun or a lamp, and gray-scale mode, where the space is illuminated by infrared camera light. Despite their HD resolution (1280x720), the video footage is of a very low quality.

The main problem consists of automatically computing the following sleep parameters for each dog, which have been recognized as important in the study:

- total amount of sleep – the number of frames in the video where the dog is asleep (i.e., lying down, eyes closed);
- sleep interval count – the number of blocks of consecutive frames where the dog is asleep in every frame;
- sleep interval length – the number of frames in a given sleep interval.

Our aim is to automatically compute these parameters for each dog by (i) localization of the dog in each frame, and (ii) classification of its state as awake or asleep. We henceforth focus on these two tasks and evaluate the performance of the system in relation to the final task (ii).

4 System Description

An overview of the system's client/server architecture is provided in Fig. 1. The input to the system is a video, and its output is a summary of the sleep parameters for that video. The video is processed by the client, and sent to the server frame by frame. The frames serve as input to a neural network, which has two main tasks: marking the dog's position, and classifying the sleep/awake state of each dog that was identified. The images are fed to the model in a sequence, which the network processes one-by-one without keeping state.

In what follows, we describe in further detail the dataset used to train the neural network, our experiments with the networks' different architectures, the post-processing methods applied to correct the network's outputs, and, finally, the calculation of the sleep parameters.

[4] The study was approved by the ethical panels of both institutions; protocol numbers: University of Salford Ethical Approval Panel - STR1617-80, CEUA/UFOP (Brazil) - 2017/04.

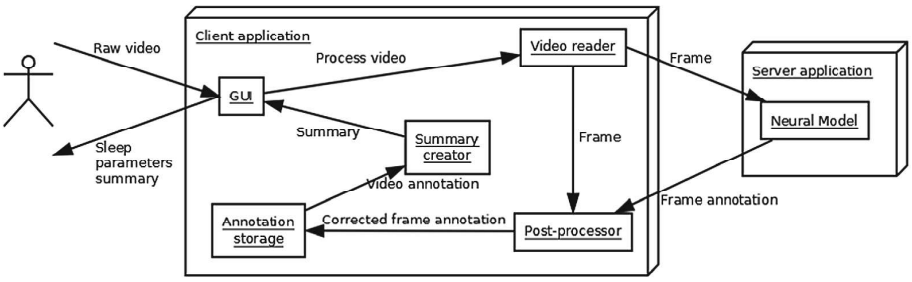

Fig. 1. System architecture.

Fig. 2. Example of frames.

4.1 The Dataset

Our training dataset consisted of 8000 frames extracted from the videos (see Fig. 2).

The obtained frame annotations included two attributes for every dog visible in the frame: (i) bounding box: an axis-aligned box surrounding each identified dog, and (ii) state of the identified dog: awake or asleep.

The annotation was performed by the first three authors independently, reaching a consensus via discussion in controversial situations (e.g., when the dog's eyes are not visible), and consulting with the last three authors who are animal experts. Frames where the dog was not clearly seen or hidden behind objects were discarded.

4.2 The Neural Network

The neural network has two tasks: (i) localization, i.e., marking the dog's position with a bounding box, and (ii) classification, i.e., marking sleep/awake state of each dog that was identified. To this end we considered two possibilities:

- Two-stage model: two distinct neural networks for the two tasks of localization and classification, packaged as one model (see Fig. 3).
- One-stage model: an end-to-end model for the detection (both localization and classification) of two types of objects: a sleeping and an awake dog.

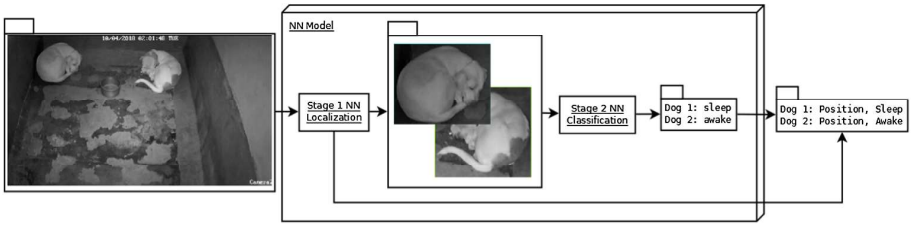

Fig. 3. Two stage model.

We decided to experiment with both types of models, comparing them using the following two criteria.

1. Intersection over union (IoU) is a standard evaluation metric in object detection. Similarly to the approach taken in [24], we calculated the widely used detection accuracy measure, mean Average Precision (mAP), based on a fixed IoU threshold, i.e. 0.5.
2. Number of unrecoverable network errors, i.e., classification errors which are impossible to recover from using the post-processing module (which will be described below). One particularly problematic error is continuous false classification of a non-moving sleeping dog.

While both approaches had comparable results with respect to the first criterion (around 0.75 mAP@0.5IoU on the evaluation set), the second approach performed much better with respect to the second criterion. Therefore, we decided to use the end-to-end architecture[5].

For object detection we used the TensorFlow Object Detection API [25].

Due to a low level of variety in training data we have chosen to use transfer learning based on state-free neural networks pretrained on the COCO dataset

[5] It should be noted that the chosen end-to-end architecture has a drawback of simultaneous detection of the same dog as sleeping and awake due to its detection of two objects (sleeping and awake dog) independently. However, this happens in very rare cases and can be overcome by using a higher confidence level for classification.

Fig. 4. Example of predicted boxes.

[26]. Initially for better performance we tried to use ssd_mobilenet_v1 [27], but it could not provide sufficient accuracy due to a number of factors, such as a small input dimension. Due to the above, we currently use faster_rcnn_resnet101 [28].

We show some samples of predicted bounding boxes of dogs in the validation set as Fig. 4 where the left column contains the model's prediction, while the right one is the ground truth as annotated by humans.

The output of the neural network consists of N tuples of the form $< x_1, y_1, x_2, y_2, R_{sleep}, R_{awake} >$, where x_1, y_1, x_2, y_2 are the bounding box coordinates, and R_{sleep}, R_{awake} are confidence scores for sleeping and awake dog respectively. This output is then transformed to $< Ind, x_1, y_1, x_2, y_2, R, Type >$, where $Type$ can be "sleep" or "awake" and $R = \max(R_{sleep}, R_{awake})$, Ind is the dog's index.

4.3 Post-processing

The main idea behind post-processing the network's outputs is compensating for possible errors produced by the network in the tasks of localization and classification. The possible errors include: double detection, random detection

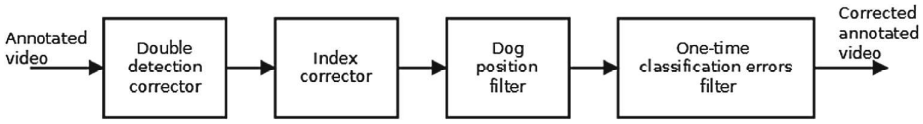

Fig. 5. Post-processing filter sequence.

order, high frequency noise in bounding box coordinates, one-time classification errors, and false-positive sleep detection (in some cases).

The post-processing module consists of a sequence of filters handling a variety of tasks, related to the above mentioned errors. The order of the filters is important due to their non-linearity. For example, it is important to eliminate double detection first, as it may result in a wrong number of detected dogs, which affects further tasks. Figure 5 presents an overview of the data flow in the post-processor module.

The input of the post-processor is a sequence of images paired with the annotations predicted by the neural network, where:

$$A = < \overline{A^{dog}}, Image > \tag{1}$$

$$\overline{A^{dog}} =< Ind, x_1, y_1, x_2, y_2, Rate, State > \tag{2}$$

and $Image$ is a $1280 \times 720 \times 3$ matrix. A_i denotes an annotated pair for frame i.

The correction tasks performed by the post-processing module as the following (in this order):

1. *Double detection correction* - based on the assumption that the euclidean distance $D(C_i, C_j)$ as in Eq. (3) between the centers C of detected boxes calculated as per (4) in the instance of double detection (between box i and j) is smaller than some ϵ, and that the probability of this situation for different dogs is quite small. The ϵ parameter is tunable.

$$D(C_i, C_j) = \sqrt{(x_i^c - x_j^c)^2 + (y_i^c - y_j^c)^2} \tag{3}$$

$$C = (x^c, y^c), x^c = \frac{x_1 + x_2}{2}, y^c = \frac{y_1 + y_2}{2} \tag{4}$$

   We calculate D on all pairs of detected boxes for the current frame, and if $D(i, j) < \epsilon$ we compare the detection rate R_i and R_j of these two boxes and delete the one with the smaller rate.
2. *Index correction* - intended for correcting random order of dog indexes Ind in the frame annotation. The index corrector works in the time domain.
   The first step of index correction is calculating the centers of bounding boxes, this data is provided by the previous step.
   The second step is calculating distance as Eq. (3) between boxes on step k and $k - 1$. At this moment we have a square matrix of distances:

$$\overline{D} = \begin{bmatrix} D(C_1^k, C_1^{k-1}) \; ... \; D(C_n^k, C_1^{k-1}) \\ D(C_1^k, C_2^{k-1}) \; ... \; D(C_n^k, C_2^{k-1}) \\ ... \quad\quad ... \quad\quad ... \\ D(C_1^k, C_n^{k-1}) \; ... \; D(C_n^k, C_n^{k-1}) \end{bmatrix} \tag{5}$$

For each column of this matrix we look for the minimal element and obtain a row of new indices $\overline{Ind_{new}}$. Then the the Ind values in frame annotations are overwritten with new values from $\overline{Ind_{new}}$.

3. *Dog position filtering.* Video can contain different high frequency noises, but the typical neural network is not totally noise invariant, therefore we should use a low frequency filter for position outputs to compensate for the shaking effect of bounding boxes on the output video.
   We use a moving average (MA) filter which s widely used as an indicator in technical analysis that helps smooth out values by filtering out the "noise" from random fluctuations. It is a trend-following, or lagging, indicator because it is based on past values. In our case we use the following difference equation:

$$P[k] = \frac{1}{n} \sum_{i=0}^{n-1} P[k-i] \tag{6}$$

   where $P = [x_1, x_2, y_1, y_2]$ are bounding box coordinates, n is the filter order (we are using $n = 5$) and all operations are element-wise.
   For an example of filtering, we can look at a plot of the x_1 coordinate for a sleeping dog in Fig. 6, where the orange line represents the non-filtered value, and the blue line is the value after filtering.The same effect applies to the values of the remaining coordinates. This transformation eliminates the jittering effect, providing the user with a more comfortable watching experience.
4. *One-time classification errors filtering.* One of the fundamental features of deep neural networks is the presence of singular points where the output value may be incorrect. Often these points can be artificially obtained by adding manually crafted noise-like signals.
   To compensate for this effect, we use two approaches. The first one is related to motion analysis. We use motion analysis techniques based on classical computer vision methods like Gaussian blur, frame delta calculation, finding contours, etc.
   For detecting movement we use a threshold based method containing the following steps: (a) crop current and previous image to dog bounding box (with coordinates from last frame); (b) convert to gray-scale; (c) calculate absolute difference between cropped images; (d) binarize image by threshold; (e) apply dilation procedure to the image for filling holes; (f) find contours on dilated image.

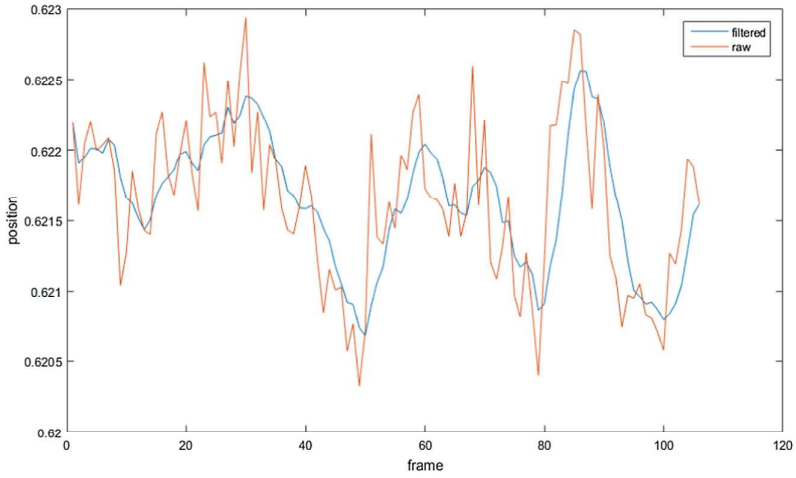

Fig. 6. Example of filter effect.

If a sufficiently large contour was found, we interpret that as evidence that the dog moved and change the dog's state to awake. This helps fixing false positives in sleeping state classification.

The second approach is filtering states. This algorithm aims to correct sequences of one state type (i.e. only sleep or only awake), that are shorter than a certain threshold. In most situations this kind of wrongly classified sequence is shorter than 3 frames. This is based on the assumption that the frequency of alternating between asleep/awake states in animals cannot be too high.

We use an approach based on remembering the currently active state of a frame sequence and switching to a new state only after seeing N frames with that state. At first glance, it may seem that this can corrupt the statistics about dog sleeping patterns, but the algorithm is symmetric in regards to states, thus the loss of the previous state's points in the beginning of a new state sequence (we had to wait N frames until toggling the state) is compensated by additional state points after the end of the sequence.

4.4 Sleep Parameters Calculation

Next we describe the calculation of the following parameters: (i) total amount of sleep, (ii) sleep interval count, and (iii) sleep interval length.

We represent a vector of dog states $State$ for dog j as follows:

$$State^j[k] = \begin{cases} 1 \text{ if } A_k[A_j^{dog}][State] = \text{“sleep”} \\ 0 \text{ if } A_k[A_j^{dog}][State] = \text{“awake”} \end{cases} \tag{7}$$

where k is the frame index.

The total amount of sleep for dog j is obtained as follows:

$$Length_j = \sum_{k=0}^{len(State^j)} State^j[k] \tag{8}$$

Sleep interval count is calculated as follows:

$$Count_j = \sum_{k=0}^{len(State^j)} \max(\Delta State^j[k], 0) \tag{9}$$

where $\Delta State^j[k]$ is defined as:

$$\Delta State^j[k] = \begin{cases} State^j[k] & \text{if } k = 0 \\ State^j[k] - State^j[k-1] & \text{if } k > 0 \end{cases} \tag{10}$$

5 Evaluation

We evaluated the system on 10 videos of total length 600 s. The video set included videos with 0–2 dogs, day/night time and different dogs and rooms. The videos were processed by the system and a testing set of 6,000 frames annotated with the system's predictions were manually checked for correctness by the authors; in controversial cases consensus was reached by discussion between the authors. The manual revision process yielded a result of 5,340 correct frame classifications.

6 Summary and Future Work

Despite dogs being a well studied species in animal science, very few works addressed so far the challenge of automatic analysis of dog behavior. In this paper we presented a system for automatic quantification of sleeping patterns of kennelled dogs, which is being currently used to measure welfare indicators in an ongoing research project. Due to the immense amount of video footage collected in the project, manual analysis is an extremely time consuming, tedious and error-prone task, to which our system, based on convolutional neural networks, provides an efficient and accurate solution. The approach presented here is based on frame vt frame analysis. One of the future research directions is to investigate more sophisticated approaches in which dependencies over time can be modelled (e.g., recurrent systems or modelling dog sleeping-states and frame dependencies using probabilistic models).

More generally speaking, behaviour analysis plays a major role in animal welfare science [29]. Our system demonstrates the potential of using neural networks for revolutionizing the way animal scientists work today. The development of automatic systems for behavior analysis has the potential for impacting the welfare of companion, farm and zoo animals, which is a problem of increasing interest for the modern society. Therefore, an important direction for future

research is making the suggested approach generalizable to other types of behavior analysis and other types of animals. Some first steps were already taken in [14].

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